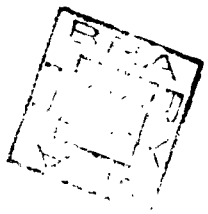
STUDIES ON CERTAIN ASPECTS OF THE CHEMICAL COMPOSITION OF FRESH WATER FISHES

ABSTRACT

KALPANA DWADASH SHRENI



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A B S T R A C T

The present work includes some interesting biochemical studies on the freshwater teleosts. The quantitative distributional patterns of fat, water, protein and ash was investigated in the flesh from different body locations of the common cat-fish, H. fossilis (Bloch). The ventral aspect of the body showed more accumulation of fat and ash than the dorsal aspect, though this dorso-ventral gradation was not evident in the protein content. The fat, protein and ash contents were also found to register an increase from the anterior to the posterior zones, both in the ventral as well as dorsal regions, of the body. The distribution of water followed an almost opposite pattern, indicating an inverse relationship with the fat and ash contents.

The various biochemical constituents of the muscle, liver and ovary of the above species showed distinct variations from season to season. The fat content of the muscle showed two peak periods of accumulation -- one during November and the other during May-July. Liver was more rich in fat than the muscle or the ovary and it was also characterized by two distinct phases of high fat content -- in May and September. In ovary, the maximum fat content was observed in June.

The lowest values of fat in both liver and ovary were observed in December. Variations in moisture content of the three tissues were found related inversely to the quantitative changes in the fat content. Protein and ash values were generally low during winter and high during summer or monsoon months. The variations in the tissue cholesterol were more or less identical to those of the fat.

The seasonal cycles of the various biochemical constituents in the three tissues of H. fossilis seemed to be governed partly by feeding and partly by the cycle of maturation and depletion of gonad. High and low values of fat and protein generally synchronized with high and low rates of feeding. There was a general build up of fat, protein and ash with ripening and a depletion with spawning. The extent of accumulation and diminution of these constituents was much greater in the ovarian tissue. The degree of hydration in tissues increased in spent fishes. Cholesterol content showed a decline in muscle and liver at peak ripeness, though in the ovary, progression in maturation resulted in a rapid increase in the quantity of this substance.

Some experimental studies were also made on fish cholesterol. In O. punctatus, a freshwater murrel, significant variations have been observed in the quantitative distribution of total cholesterol from one organ to the other. Liver, brain and spinal cord were fairly rich in cholesterol, while the muscle tissue was the poorest in this respect. The total liver cholesterol level of this species was found

to be markedly influenced by the age of the fish. A substantial rise in the concentration was evident with increasing age up to a maximum when the fish attained an age of 4^{+} years but significant fall occurred beyond this age. These changes were thought to be a manifestation of aging and a net result of variations in growth rate, diet and sexual cycle of the fish.

A comparative quantitative study of the distribution of total cholesterol in the brain, spinal cord and eggs of some freshwater teleosts indicated that the cholesterol content was highest in the nervous tissues of the active murrels but was relatively low in herbivorous carps. The spinal cord of all the species analysed contained more concentration of cholesterol than the brain. The ripe, unspawned teleostean eggs were also found to be fairly rich in this substance, though quantitative variations were observed from one species to another. The distribution of cholesterol in the nervous tissues and the eggs, in general, seemed species specific.

Liver cholesterol level of the major carp C. mrigala, has been found to be markedly influenced by the cycle of maturation and depletion of its gonad. The highest value of cholesterol was noted during the recovering phase. Advancement in maturation was accompanied by a depletion in the liver cholesterol and the minimal value was reached at the time of peak ripeness. These changes in the concentration pattern of liver cholesterol seemed to be related to variations in the

cholesterol metabolism of the fish, necessitated, besides other factors, by the demand for sex hormones.

The cholesterol level of fish was significantly influenced by starvation and showed a marked response to different types of diets. In H. fossilis, the cholesterol level decreased with starvation in liver but in brain, after registering an initial fall, it showed a distinct rise. These changes have been attributed to the changes in the rate of cholesterol synthesis and metabolism. The cholesterol level of the blood of another cat-fish, C. batrachus, declined with an increased uptake of the carbohydrate and protein diets but showed a distinct rise with cholesterol (fat) diet. The liver cholesterol level of this species, on the other hand, registered an initial increase with the protein and carbohydrate diets, but declined substantially when higher doses of these diets were offered to the fishes. Increased intake of cholesterol diet induced an overall fall in the liver cholesterol level. An inverse relationship was noted between the liver and the blood cholesterol levels of the fishes fed on cholesterol diet. An evidence of a feedback mechanism operating in the liver of C. batrachus was thus obtained.

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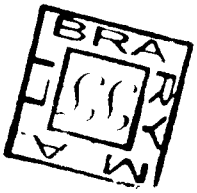

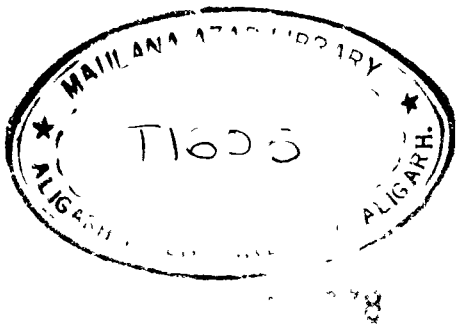

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By

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in candidature for the degree of

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C O N T E N T S

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GENERAL INTRODUCTION AND HISTORICAL RESUME

Fish meat is one of the best sources of animal protein and other indispensable dietary constituents. The dietary qualities of fish with all the constituents necessary for growth and maintenance of the body have made it an important item of food all over the world. Fish meat also constitutes a valuable balanced food for domestic animals and is considered to be virtually indispensable for pigs and poultry. Furthermore, fish oil represents a good source of calories and vitamins.

The importance of fish as food was much better realized after the first world war when an acute shortage of protein food occurred. The need for a comprehensive resource management as well as the wise and efficient utilisation of fish became paramount in the face of mounting population pressures. It was felt that an adequate knowledge of the chemical composition and nutritive value of the various species is essential, if fish is to be appreciated as an important item of diet. The application of this knowledge in fish canning and processing industries became obvious. The recognition of the importance of this problem diverted the attention of fishery scientists and chemists all over the world to the investigation of the various aspects of the chemical biology of fish.

In recent years, well-planned world-wide surveys and research projects have been launched by FAO, with the cooperation of the WHO, to estimate the extent of protein malnutrition in developing countries and the consumption of more fish is being encouraged to supplement such deficiencies. In India, a number of similar programmes have been launched, in collaboration with several International agencies, to give reappraisal and reorientation to the development of fisheries on a rational basis, for the benefit of the undernourished sections of the population.

A useful amount of data has been collected in the past in various countries on the chemical composition of different fish species. The first classical study of this nature was carried out by Atwater (1888) on 53 different species of American food fishes.

Other important contributors in the field include Johnstone (1915, 1917, 1918a, b, 1920); Clark and Almy (1918); Dill (1921); Valenzuela (1928); De Clercq (1932); Carteni and Aloi (1934); Salgues (1934); Lovern (1932a, b, 1934a, b, 1935, 1937, 1938a, b, 1942, 1950, 1958); Lovern and Wood (1937); Jowett and Davies (1938); Kondo et al., (1941); Bailey (1942); Reay et al., (1943); Van Wyk (1944); Levanidov (1950); Bailey et al. (1952); Lühmann (1953); Tsuchiya et al. (1953); Vinogradov and Odum (1953); Bramsnaes et al. (1954); Stansby (1954); Sulit et al. (1954); Karrick et al. (1956); Love (1957, 1960); Idler and Bitners (1958, 1960); Meyer (1958); Kochi and Era (1959); Marinkovic and Zei (1959); Thompson (1959); Thurston et al. (1959); Jacquot (1961); Thurston (1961, 1962); Vasil'yeva et al. (1961); Parker and Vanstone (1966); Groves (1970).

In addition, a number of investigations have been compiled in the form of nutritional tables by many authors (McCance and Widdowson, 1940; Taylor and Macleod, 1949; Vinogradov, 1953; Kuppuswamy et al. 1958; Love et al., 1959). Several official documents of the United Nations (FAO, 1949, 1954) and other organisations (Nilson, 1959) also provide valuable information on the subject. A bibliography containing 436 references has been compiled by Vander Rijst (1950). A review by Tarr (1958) and a symposium on 'Fish Biochemistry' held under the auspicies of the Biochemical Society (Williams, 1951) give useful information on the subject.

A recent book on the 'Chemical biology of fishes' by Love (1970) forms another interesting and important contribution to the subject.

Numerous papers have also appeared dealing with the individual chemical constituents of fish muscle (Yagana, 1975) but a review of these is beyond the scope of the present writing.

Cholesterol, one of the primary cell constituents, has been the centre of investigations for the last several decades, largely because of its role in certain coronary diseases in human subjects. The knowledge of cholesterol metabolism and biosynthesis is restricted almost exclusively to higher vertebrates.

Investigations on fish cholesterol have been related mainly to its distribution in various tiesues, its synthesis and relationship with feeding, maturation and spawning (Love, 1970).

The distribution of cholesterol in the dark and ordinary muscles and several other fish tissues was studied by Namiki (1933), Bligh and Scott (1966), Igarashi et al. (1957), Katada et al. (1959, 1960), Zama (1963), Shimma and Taguchi (1964a, b).

The cholesterol content in the brain and nervous tissues of fish was investigated by Ogino and Konno (1950), McColl and Rossiter (1952a, b), Kawakita (1956) and Ananichev (1961).

Plasma and serum cholesterol levels have also been estimated in many species of freshwater and marine fishes (Field et al., 1943; Phillips, 1958; Phillips et al., 1957; Morris, 1959; Sulya et al., 1960; Shell, 1961; Tamura et al., 1962; Hunn and Robinson, 1966; Lauter et al., 1968).

McCartney (1965) has described the influence of age and sex on the total serum cholesterol level of the brown trout. In a later publication (McCartney, 1966), monthly variations in the total serum cholesterol level of this fish were also discussed.

The changes in the tissue and serum cholesterol levels of several fish species accompanying sexual maturation and spawning have been investigated by Channon and El-Saby (1932), Idler and Bitners (1958, 1960), Idler and Tsuyuki (1958) and Robertson et al. (1961a, b).

Irvine et al. (1957) have reported the effect of dietary phospholipid and cholesterol on the temperature resistance of the goldfish. The effect of the essential oils of garlic and onion on alimentary hyperlipemia has

also been studied by Bordia et al. (1975).

In India, although several studies have been made in the past on the chemical composition of fishes, the information is still meagre, considering the variety of fishes that are found in our inland and coastal waters. Most of the earlier studies were designed from the nutritional point of view.

Interesting data on the chemical composition and nutritive value are available on fishes from the coastal waters of India. Niyogi et al. (1941) have analysed thirteen species of dried and five species of undried marine fishes of the Bombay coast. Similar study has been made on several species of fish and prawn from the Bombay and Konkan coasts (Appanna and Devadatta, 1942). Setna et al. (1944) gave an account of the chemical composition and nutritive value of representative fishes from the commercial catches off the Bombay coast. Another important work on the Bombay fishes was that of Patakoot et al. (1950) who analysed about thirty-two common species.

Fishes of the Madras coast have been analysed for their chemical composition and calorific value by several workers (Seshan, 1940; Chari, 1948).

On the freshwater fishes, Saha and Guha (1939, 1940) made a detailed chemical observation of twenty-four different varieties of food fishes from the Bengal region while Basu and De(1938) reported on the nutrive value of some commercially important freshwater species of fish. Airan (1950) estimated the protein and mineral contents of several species of

freshwater fishes from Kolhapur. The protein value of a number of representative vertebrates, including fishes, has been studied by Alexander (1956). Natarajan and Sreenivasan (1961) made a comparative study of a large number of freshwater fishes from the Bhavanisagar reservoir. Bhatt et al., (1962, 1963) gave an account of the mineral contents of some important freshwater fishes from the fresh waters of Gujarat, Maharashtra and Bihar states. The chemical composition of certain commercially important fishes found in the Jaisamand lake (Rajasthan) has been investigated by Sharma and Simlot, (1971).

Comparative studies on the gross chemical composition of the red and white muscles have also been carried out on some freshwater and marine fishes of India (Alexander, 1955; Bhushana Rao, 1965).

The amino acid make up of the muscle has been described for several freshwater and marine fish species of India (Kulkarni, 1953; Master and Magar, 1954; Ambe and Sohonie, 1957; Valanju and Sohonie, 1957 a, b; Velankar and Govindan, 1957; Venkataraman and Chari, 1957; Bose et al., 1958; Joshi et al., 1958; Durairaj, 1961).

Seasonal variations in the chemical composition of fish from Indian waters have also been studied by a few workers (Hornell and Naidu, 1924; Sekharan, 1949, 1955; Chidambaram et al., 1952; Venkataraman and Chari, 1951, 1953; Vasavan et al., 1960).

However, little systematic work has been done on the cholesterol content of Indian fishes and physiological studies are relatively few.

Joshi and Magar (1955) studied the quantitative distribution of various lipids, including cholesterol, in the tissues of some marine fishes and Das (1965) has referred to the influence of age on the blood cholesterol of the carp, Catla catla.

Of late, a series of interesting papers have appeared on the various aspects of the chemical composition of freshwater fishes from the north Indian environment (Jafri, 1968a, b, 1969, 1973; Jafri and Khawaja, 1968, 1970; Jafri et al., 1964; Jafri and Qasim, 1965, 1966; Khawaja, 1966; Siddiqi and Siddiqi, 1965; Siddiqui and Siddiqui, 1968). Yagana (1975) has recently worked out the detailed chemical composition of the cat-fish, Clarias batrachus. Some information has also been obtained on the seasonal variations in the cholesterol content of the tissue and blood plasma of several north Indian freshwater fishes (Siddiqi, 1966c; Siddiqui, 1972). The existence of a feedback mechanism of cholesterol operating in the liver of the freshwater murrel, Ophicephalus punctatus, has been pointed out by Siddiqi (1966a). These authors have emphasised the need for more detailed investigations, on these lines, especially on fishes from the tropical environment.

Studies on certain aspects of the chemical composition of freshwater fishes were, therefore, initiated by the present author as a continuation of the earlier work on the subject and the results obtained have been presented here in the form of this thesis. The entire work has been conveniently divided into two parts.

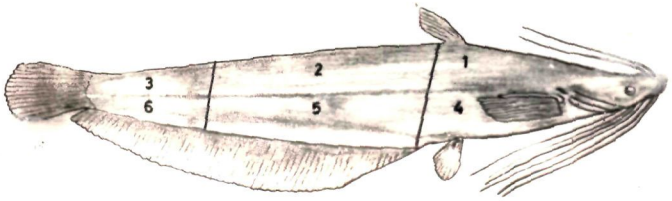
Part I deals with the studies on the distributional patterns of fat,

water, protein and ash in the flesh from different body locations of the common freshwater cat-fish, Heteropnuestes fossilis (Bloch.). It also includes investigations concerning the seasonal variations in the chemical composition of the various tissues of this species.

Part II is devoted to the detailed studies carried out on fish cholesterol. It deals with the distribution of total cholesterol in the normal organs of the freshwater murrel, Ophicephalus punctatus Bloch. and in the eggs and nervous tissues of certain freshwater teleosts. Variations in the total liver cholesterol level of the major carp, C. mrigala during maturation and the influence of age on the liver cholesterol level of O. punctatus has also been described. In addition, the effects of starvation and diets on the tissue cholesterol levels have been studied in the cat-fishes, Heteropnuestes fossilis (Bloch.) and Clarias batrachus (linn.), respectively.

The present investigation on cholesterol assumes significance since it provides a physiologically oriented experimental account in some of the commonest freshwater fishes from the northern Indian environment.

Fig. 1 Showing different regions and zones of the body of H. fossilis selected for muscle sampling.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PROCEDURE AND METHODOLOGY

A. TECHNIQUES OF TISSUE SAMPLING

I. For the study of the fat, water, protein and ash distribution pattern in the flesh of the cat-fish, Heteropnuestes fossilis (Bloch.):

Fishes of a particular size (24 cm total length) were brought to the laboratory in fresh condition. The body of each individual was divided into two horizontal regions along the lateral line, each of these being further divided into three vertical zones, head, trunk and tail, as shown in Fig. 1. The skin and bones were then carefully separated out from the muscle samples. All the determinations were made in triplicate.

II. For the study of the seasonal variations in the biochemical composition of the cat-fish, Heteropnuestes fossilis (Bloch.):

Fish samples for the study of seasonal variations in the biochemical composition of tissues (muscle, liver and gonad) were obtained at regular monthly intervals over a period of one year (from February, 1974 to January, 1975). To avoid any difference due to size heirarchy, adult fishes of a definite size-range were used for the collection of tissue samples.

Fishes were sexed and the data for each sex kept separately. All the determinations were made in triplicate.

III. For the study of the quantitative distribution of cholesterol in the normal organs of the murrel, Ophicephalus punctatus Bloch.:

Adult fishes of a definite size-range (20-21 cm total length) were brought to the laboratory, from a few selected ponds, in live condition. These were then decapitated and their tissues (organs) dissected out as quickly as possible. A weighed amount of each tissue (0.1 g) was taken for chemical analysis. For each organ triplicate estimations were made.

IV. For the study of the quantitative distribution of cholesterol in the brain and spinal cord of some freshwater teleosts:

Adult fishes were obtained from the local fish market. The brain and spinal cord were dissected out from each specimen and the required amount of tissue sample rapidly weighed. For all fishes, analyses were carried out on the whole brain. All determinations were made in triplicate.

V. For the study of the quantitative distribution of cholesterol in the eggs of some freshwater teleosts:

Fully ripe and almost running fishes were procured from the local fish market and their ovaries dissected out. The eggs were then quickly removed from the ovaries and sampled for cholesterol estimation. For each species, duplicate determinations were made and the mean values calculated. The egg diameter records were made on the samples fixed in 10 % formalin for about an hour.

VI. For the study of the total liver cholesterol of the carp, Cirrhina mrigala (Ham.) during maturation:

Sexually mature fishes of 45 to 50 cm total length were obtained from the local fish market during different seasons of the year. These were dissected out and their maturity stages arbitrarily established on the basis of size, colour and weight of the gonad, following the scheme as suggested by Qayyum and Qasim (1964) for O. punctatus. The fishes were classified into four distinct maturity stages, viz., recovering (stage I), ripening (stage II), ripe (stage III) and spent (stage IV). Their liver were then taken out and cleaned by gently blotting with filter paper. A weighed amount of liver tissue was taken from each individual for cholesterol estimation. The two sexes were analysed separately. At least three determinations were made for each maturity stage and the mean values calculated.

VII. For the study of the influence of age on the total liver cholesterol level of the murrel, Ophicephalus punctatus Bloch.:

Random samples of live O. punctatus were procured from a local pond and kept in the laboratory in continually aerated aquaria. The fishes were weighed and measured to the nearest millimeter. They were then arranged into age-estimates, corresponding to year classes 0^+ to 6^+, on the basis of a scheme suggested by Qasim and Bhatt (1966) for this species. At least three individuals from each age group were then killed by decapitation, their livers dissected out and soaked on to a filter paper to remove any adhering fluid or blood. Weighed amount of liver sample was taken from each individual for chemical analysis. Since

sufficient number of specimens of each sex could not be obtained for all the age groups, the two sexes were not analysed separately. Similarly, to avoid any seasonal or environmental differences, the entire study was completed within a fortnight and all fishes were obtained from the same environment.

VIII. For the study of the influence of starvation on the total tissue cholesterol content of the cat-fish, Heteropnuestes fossilis (Bloch.) :

Live H. fossilis of 17-25 cm total length were selected for the present study. The fishes were kept in an aquarium (95 x 35 x 45 cm), supplied with water at a temperature of 14 $\pm$ 2^{o}C. The fishes were tagged so as to record the decline in their weights during the successive periods of starvation. For tagging, modified collar tags were used which consisted of a rectangular plate of ivory paper (9 x 6 mm) covered over by a water proof, transparent, self-adhesive tape. The plate was pierced by a filamentous wire which formed a loop around the trunk region adjacent to the dorsal fin. Three fishes were taken out each time at the intervals of every 10 days. Equal number of controls were maintained to access the normal values of cholesterol. The starvation was continued over a total period of 50 days.

At the time of sampling, the fishes were taken out from the aquarium, weighed and measured to the nearest millimeter. They were killed by a sharp blow on the head, their tissues (liver and brain) carefully removed and processed for chemical analysis.

IX. For the study of the influence of diet on the total blood and liver cholesterol level of the cat-fish, Clarias batrachus (Linn.):

Live C. batrachus of a particular size-range (20-24 cm total length) were brought to the laboratory and kept in aerated aquarium (95 x 35 x 45 cm). Different concentrations of three different diets, namely, protein, carbohydrate and fat (cholesterol), were used to feed the fishes. Each set of experiments were conducted in triplicate. The protein diet consisted of the egg albumin. Three concentrations, 0.25 ml, 0.50 ml and 0.75 ml, of the egg albumin were fed two times, at the time interval of 24 hours, to three groups of fishes kept in separate aquaria. A group of control fishes were also maintained in order to assess the normal cholesterol level and no special diet was given to these fishes. After 48 hours of the first intake of diet, the fishes were dissected out.

For the carbohydrate diet, a 100 % solution of glucose was prepared. Different quantities of this solution, containing 0.2, 0.4, 0.6 and 1.0 g of glucose, respectively, were given to different groups of fishes. As in the case of protein diet, after 24 hours, the fishes were again fed with the same quantities of glucose diet and were dissected out after 48 hours of the first intake of diet.

For the fat diet, chemically pure cholesterol was obtained. 5 mg, 10 mg, 15 mg and 20 mg doses of cholesterol were fed to four different groups of fishes. The fishes were refed after 24 hours and dissected out after 48 hours of the first feeding.

All the feedings were carried out in the morning time. Care was taken to ensure that only healthy fishes were included in the samples.

At the time of sampling, fishes were taken out of the aquaria. They were weighed on a sensitive balance. The blood was obtained by severing the tail with a sharp knife and collected in test tubes which had earlier been rinsed with anti-coagulant sodium oxalate solution (0.2%). 1 ml of the blood was mixed with about 8 g of sodium sulphate (anhydrous) and kept in an oven at 100°C for drying. Liver from each specimen was carefully dissected out. 0.1 g of liver was mixed with 8 g of anhydrous sodium sulphate in a mortar and kept likewise in an oven at 100°C.

B. METHODS OF ESTIMATIONS

I. Moisture:

Moisture in various tissues was determined according to the standard technique (A.O.A.C., 1960). A known amount of fresh sample (5-10 g) was taken in a weighed silica crucible and kept in an electrial oven maintained at 100°C for 14-16 hours till all the traces of moisture were completely expelled. The crucible was then cooled in a dessicator and reweighed. The process was repeated several times till the crucible showed a constant weight. From the values thus obtained, moisture in fresh tissue was calculated.

II. Ash:

For the determination of ash content, a known amount of fresh tissue sample (5-10 g) was taken in a weighed silica crucible and kept

in an oven at 100°C (A.O.A.C., 1960). It was then ignited till it became completely white and free from carbon. The crucible was reweighed after cooling. The whole process was repeated till a constant weight was obtained. The percentage of ash in each tissue was calculated on fresh weight basis.

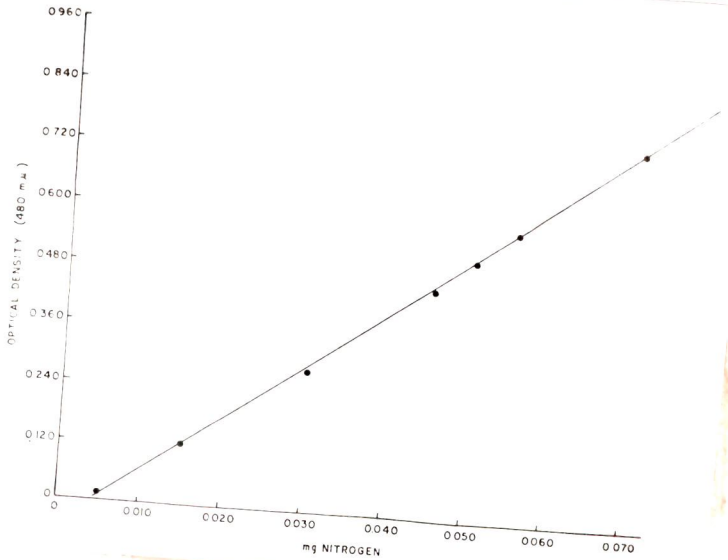
III. Total fat:

The determination of total fat was carried out in a continuous soxhlet extraction apparatus using petroleum ether (40-60°C B.P.) as solvent. Fresh tissue samples were dried in an oven at 100°C, mixed and ground thoroughly. Weighed quantity of each tissue sample was taken in Whatman extraction thimble and plugged carefully with cotton wool. Extraction was carried out for about 10-12 hours. The distillation of solvent from the fatty extract was done by keeping the receiving flask on a water bath. The flask was then kept in an oven to remove the final traces of the solvent, cooled in a dessicator and weighed. The increase in the weight of the flask gave an index of fat from which the percentage of fat was calculated on a fresh weight basis. To ensure complete fat extraction from the tissue, the process was repeated till a constant weight was obtained and no further increase in the weight of the receiving flask observed.

IV. Protein:

The estimation of protein in different tissues was made by slightly modifying Wong's (1923) micro-kjeldahl technique. A known amount of the fresh tissue (0.1 g) was digested in 5 ml of extra pure (nitrogen free) 1:1

Fig. 2 Calibration curve for the estimation of total nitrogen.


0.960
0.840
0.720
0.600
0.480
0.360
0.240
0.120
0
OPTICAL DENSITY (480 mμ)
0
0.010
0.020
0.030
0.040
0.050
0.060
0.070
mg NITROGEN

sulfuric acid. Potassium persulphate was used as an oxidizing agent. The digestion, which converts all the nitrogenous substances into ammonium sulphate, was carried in an Gallenkamp's electrical digestion unit and was continued till the digestion sample became water clear. The digested sample was diluted to 50 ml in a volumetric flask. Known aliquotes of this solution were taken in separate tubes and then nesslerized by adding Koch and McMeekin's Nessler's reagent (Hawk et al., 1954). The mercuric iodide contained in the Nessler's reagent combines with the ammonium sulphate of the digested solution producing a brown coloured solution of oxy-dimercuric iodide $(OHg)_2 NH_2I$. The intensity of the colour developed is proportional to the amount of ammonium sulphate present in the solution. The colour intensity was read against a blank solution on a Bausch and Lomb Spectronic 20 Spectrophotometer at 480 mμ wave length.

The readings obtained for various samples were then read against a standard calibration curve which was prepared by plotting the readings of serially diluted solutions of ammonium sulphate (see Fig. 2). The standard solutions of ammonium sulphate always contained known amounts of nitrogen. This gave a direct reading of the amount of total nitrogen present in the various samples. The percentage of protein was calculated by multiplying the total nitrogen values with the protein factor (6.25).

V. Cholesterol:

Total cholesterol in all the tissues was estimated using the method of Reinhold and Shiels as described by Hawk et al. (1954). The details of the procedure followed was as follows:

1. Method of extraction:

A weighed amount (0.1 g) of tissue was transferred to a small mortar containing about 8 g of pure anhydrous sodium sulphate and mixed uniformly. It was then dried in an oven at 100°C for about 12 hours, cooled in a dessicator, pulverized and transferred carefully into a Whatman thimble and cotton plugged. The thimble containing the dried tissue sample was placed in a soxhlet assembly fixed on to a water bath. Extraction was carried out, using about 50 ml of redistilled choloroform as a solvent, for exactly 3 hours. The extract was allowed to cool and transferred quantitatively with rinsings to a 25 ml volumetric flask, made up to volume with chloroform and mixed well.

2. Method of Photometric estimation:

Total cholesterol in the extract was determined photometrically by the Liebermann Buchard reaction with acetic anhydride and sulphuric acid.

10 ml of the chloroform extract, obtained by the method described above, was transferred to a dry test tube. To this was added 2 ml of the freshly prepared acetic anhydride-sulphuric acid reagent. A 10 ml standard solution of cholesterol in chloroform, containing 0.8 mg of cholesterol per 10 ml, was treated in the same way. Similarly, a blank control tube was prepared by treating a 10 ml portion of chloroform with the acetic anhydride-sulphuric acid reagent. After proper mixing, the three tubes were kept in darkness in a water bath at 25°C for exactly 30 minutes for colour development. The colour intensity of the

experimental and standard solutions was read on a Bausch and Lomb Spectronic 20 Spectrophotometer after setting the instrument with a blank solution at 660 mμ wave length. The amount of total cholesterol in the sample was calculated using the following formula:

$$\frac{\text{Density of unknown}}{\text{Density of standard}} \times 0.8 \times \frac{100}{4} = \text{mg of cholesterol per 10 g of tissue.}$$

From the value thus obtained, the amount of total cholesterol per 100 g of tissue was calculated. In the blood, cholesterol was calculated as mg per 100 ml.

C. STATISTICAL ANALYSIS

The statistical analyses of the data for the various sets of studies were made according to the methods as given by Snedecor (1959).

PART I

CHAPTER - I

FAT, WATER, PROTEIN AND ASH DISTRIBUTION PATTERN IN THE FLESH OF THE COMMON CAT-FISH, HETEROPNUESTES FOSSILIS (BLOCH.)

INTRODUCTION

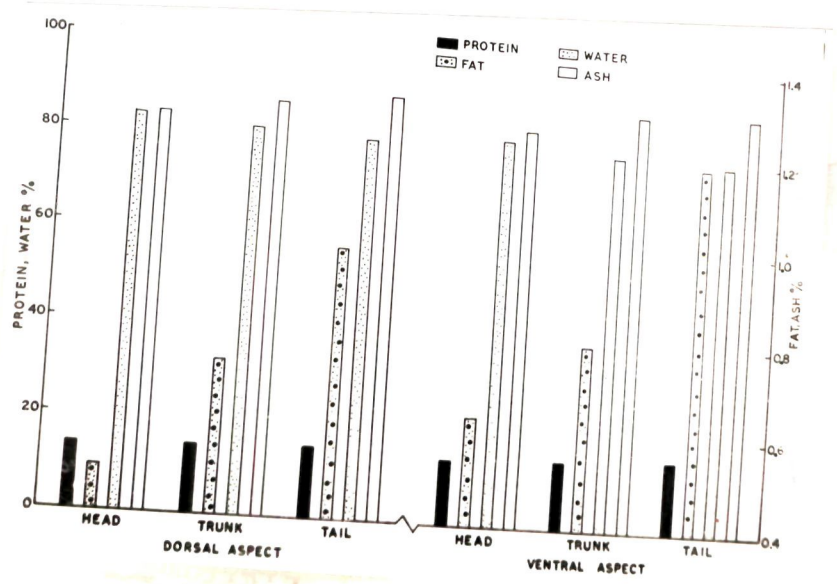
It is well known that the biochemical constitution of fish is effected by many factors like size, sex, species, maturity, feeding, environment and season, etc. In addition, individual variations and variations with different anatomical locations have also been recorded (Love, 1970). Variations in the chemical composition and nutritive value of muscle from different parts of the individual fish have been emphasised by several workers in the past (Brandes and Dietrich, 1953a, b; Alexander, 1955; Olley and Lovern, 1960; Thurston and MacMaster, 1960; Mannan et al., 1961; Karrick and Thurston, 1964; Jafri, 1973).

The present chapter describes the variations in the total fat, water, protein and ash contents of the flesh of a popular cat-fish, Heteropnuestes fossilis (Bloch.), from different body locations.

MATERIALS AND METHODS

Details of the methods of sampling and various estimations were the same as described earlier under 'Procedure and Methodology'.

Fig. 3 Histograms showing the distribution pattern of fat, water, protein and ash in the flesh of H. fossilis (Bloch.).


PROTEIN
FAT
WATER
ASH
PROTEIN, WATER %
FAT, ASH %
100
80
60
40
20
0
1.4
1.2
1.0
0.8
0.6
0.4
HEAD
TRUNK
TAIL
DORSAL ASPECT
HEAD
TRUNK
TAIL
VENTRAL ASPECT

RESULTS AND DISCUSSION

The data obtained for the distribution of fat, water, protein and ash contents of the muscle from different body regions of H. fossilis have been presented in Table 1 and Fig. 3.

As would be evident from the data, the various chemical constituents of the fish showed interesting distributional pattern in the flesh from different body regions of this species.

I. Variations in the fat content:

Fat values observed in the muscle of different locations of H. fossilis have been given in Table 1 and plotted in Fig. 3.

The muscle from the various zones of the ventral portion of the body showed higher values of fat than the muscle from the dorsal body zones (Table 1). Higher lipid values observed in the ventral region of the fish have been reported to be characteristic of many fish species like herring, Clupea harengus (Brandes and Dietrich, 1953a), the siscowet trout, Salvelinus namaycush siscowet (Thurston, 1962), as also of certain Indian species, namely, Arius dussumieri, Ophicephalus striatus (Alexander, 1970), and Wallago attu (Jafri, 1973). Further, it has been observed that in the ventral region, the percentage of fat increased from the head (zone 4) to the tail (zone 6) region and this has been found to be consistent with the observations of Jafri (1973) on W. attu. In the dorsal region, as well, the percentage of fat was found to increase

from the anterior (zone 1) to the posterior(zone 3) location. Higher fat accumulation recorded in the caudal region, both in the dorsal as well as in the ventral aspects, might be associated with a greater demand of energy for muscular activity of the tail during swimming, which involves swift lashing of this region of the fish body. Similar observations were made in certain other fishes (Alexander, 1970; Jafri, 1973).

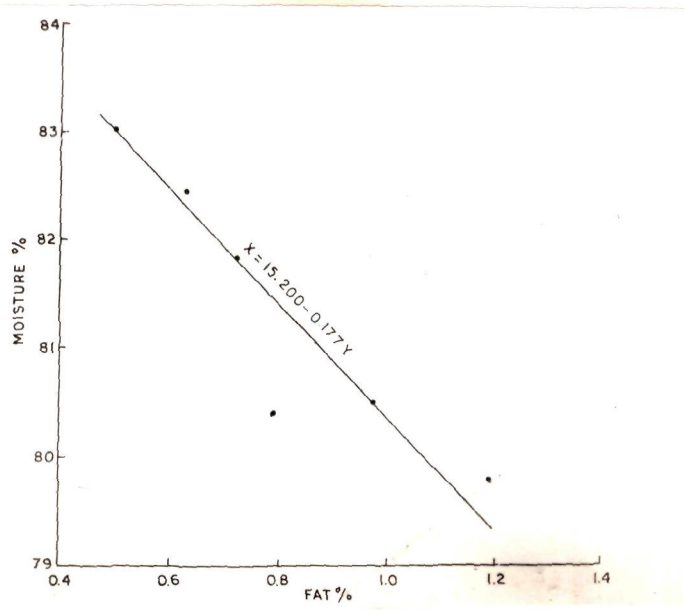
II. Variations in the moisture content:

It would be evident from the data given in Table 1 that there was a definite dorso-ventral gradation in the moisture content of the body. The moisture values were slightly higher in the dorsal region than in the ventral region of the fish. The highest moisture percentage was recorded in the dorso-ventral aspect of the head region while the lowest values were recorded in the caudal peduncle region. Of all the six regions analysed, the highest moisture was found in the dorsal aspect of the head region (zone 1) and the lowest in the ventral side of the tail region (zone 6).

The percentage of water maintained an inverse relationship with the percentage of fat in the muscle. Similar relationship was pointed out in fish muscle by several other workers (Brandes and Dietrich, 1953a, b, 1956, 1958; Iles and Wood, 1965).

The fat/water relationship in the muscle of H. fossilis could be explained through the following regression equation:

Fig. 4 Relationship between fat and water distribution pattern in the flesh of H. fossilis (Bloch.)


84
83
82
81
80
79
MOISTURE %
0.4
0.6
0.8
1.0
1.2
1.4
FAT %
X = 15.200 - 0.177 Y

$$X = 15.200 - 0.177\ Y$$

Where, X was the percentage of fat, and Y was the percentage of water.

The correlation coefficient for this relationship was found to be - 0.924, significant at 1 % level.

The fat-water line for the muscle of H. fossilis has been plotted in Fig. 4.

III. Variations in the protein content:

The muscle protein content did not show any marked variations between the dorsal and ventral regions of the body. However, a marked increase could be seen from the head to the tail region of the fish (Table 1, Fig. 3).

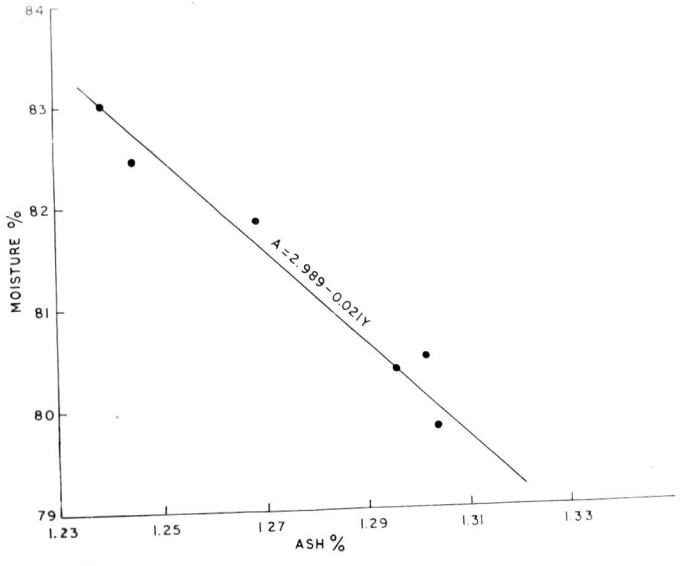
IV. Variations in the ash content:

The variations in the muscle ash content were not well marked (Table 1) but declining values of ash were observed with increase in the degree of hydration of the muscle in various regions of the fish.

The relationship between the ash and water has been represented in Fig. 5 and could be expressed by the following equation:

$$A = 2.989 - 0.021\ Y$$

Where, A was the percentage of ash, and Y was the percentage of water.


84
83
82
81
80
79
MOISTURE %
A=2.989-0.021Y
1.23
1.25
1.27
1.29
1.31
1.33
ASH %

The value of correlation coefficient, 'r', for the above relationship was found to be -0.927, significant at 1% level of probability ($P < 0.01$).

The present investigation on H. fossilis thus indicate that any increase in the proportion of any one of the major biochemical constituents of fish muscle is generally accompanied with a simultaneous decrease in that of the others, the sum of these remaining almost relatively constant.

SUMMARY

The quantitative distributional pattern of fat, water, protein and ash has been studied in the flesh of the various zones corresponding to the dorsal and ventral regions of the body of a common cat-fish, H. fossilis (Bloch.). The ventral aspect of the body showed more accumulation of fat and ash than the dorsal aspect while this dorso-ventral gradation was not marked in the case of protein content. The fat, protein and ash contents were also found to register an increase from the anterior to the posterior zones, both in the ventral as well as dorsal regions, of the body. The distribution of water followed an almost opposite pattern, indicating an inverse relationship with the fat as well as with the ash contents.

CHAPTER - II

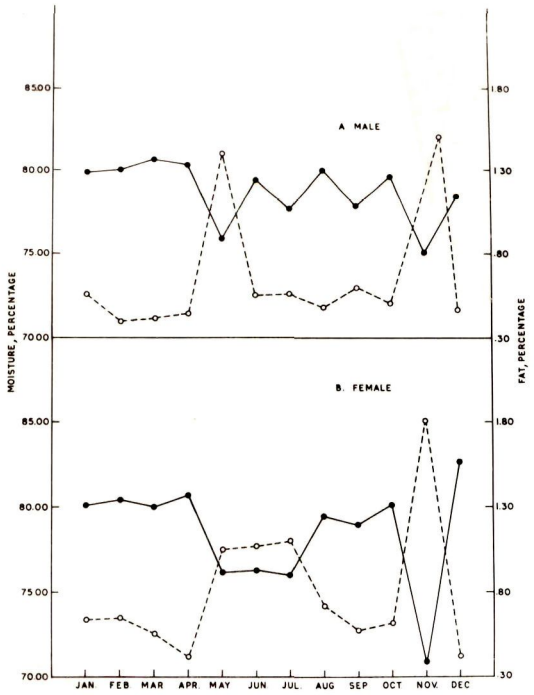
SEASONAL VARIATIONS IN THE CHEMICAL COMPOSITION OF THE CAT-FISH, HETEROPNUESTES FOSSILIS (BLOCH.)

INTRODUCTION

It has been fairly well established that in many fishes, both from freshwater and marine environments, marked changes occur in the levels of the chemical constituents of tissue from season to season. Many workers in the past have recorded the effects of seasonal variations on the chemistry of fish tissue and have observed that the effects include not only the environmental influences but also those of growth, feeding, maturity and spawning (Milroy, 1908; Clark and Almy, 1918; Dill, 1921; Bruce, 1924; Bull, 1928; Lovern and Wood, 1937; Bailey et al, 1952; Idler and Bitners, 1958; Love, 1957, 1970). However, to quote Jacquot (1961): "The significance of seasonal variations is complex and it is almost impossible to distinguish surely between the effect of many factors, which play a part". Undoubtedly, the mechanism of a number of variations are completely unknown. The underline idea of the present study is to describe the phenomenon of seasonal variations in the tissues of the cat-fish, Heteropnuestes fossilis (Bloch.), from the freshwater environment

Fig. 6 Seasonal variations in the moisture and fat contents of the muscle of H. fossilis.

●——— , Moisture; o - - - -, Fat.


A. MALE
B. FEMALE
MOISTURE, PERCENTAGE
FAT, PERCENTAGE
85.00
80.00
75.00
70.00
1.80
1.30
.80
.30
JAN. FEB. MAR. APR. MAY JUN. JUL. AUG SEP OCT NOV. DEC

of north India, and to correlate these with some such factors like feeding and maturation cycles of the fish.

MATERIALS AND METHODS

The methods of collection of tissue samples and various estimations were the same as described under 'Procedure and Methodology' (pages 9-10). As a rule, maturity was regarded as the most important variable influencing the levels of the various biochemical constituents of fish and, therefore, the gonad condition of the fish in each month was arbitrarily established on the basis of size, shape and colour of the gonad, following the scheme as suggested by Qayyum and Qasim (1964). Similarly, a record of the gut content analyses was maintained for each month's sample to assess the seasonal feeding rhythm. The gut content analysis was carried out using the conventional number method. Since enough testicular tissue could not be obtained for chemical analysis in each month, the variations in male fish could only be studied in muscle and liver tissues.

RESULTS AND DISCUSSION

I. Variations in fat content:

Seasonal variations were observed in the fat content of the muscle, liver and ovary of *H. fossilis*.

(a) Muscle:

Muscle fat content in both the sexes showed marked fluctuations from season to season. From the values given in Table 2 and plotted in

Fig. 7 Seasonal variations in the moisture and fat contents of the liver of H. fossilis

●———— Moisture ; o-----, Fat

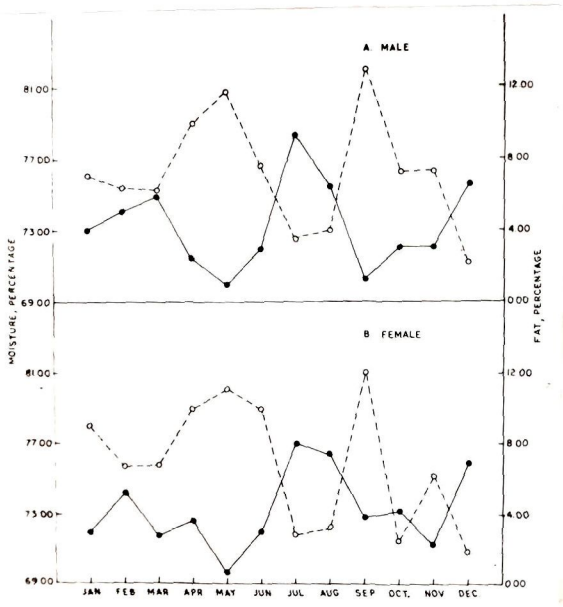

A. MALE
B. FEMALE
81.00
77.00
73.00
69.00
12.00
8.00
4.00
0.00
MOISTURE, PERCENTAGE
FAT, PERCENTAGE
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT.
NOV
DEC

Fig. 6, it would be evident that fat in muscle had two peaks of accumulation -- during November and May. Low values were observed from December to April and between the two peaks. The seasonal trends in the two sexes were more or less identical, except that in female the first peak of fat accumulation occurred for an extended period (May to July). In most of the months the females contained relatively more fat in the muscle than the males (Table 2).

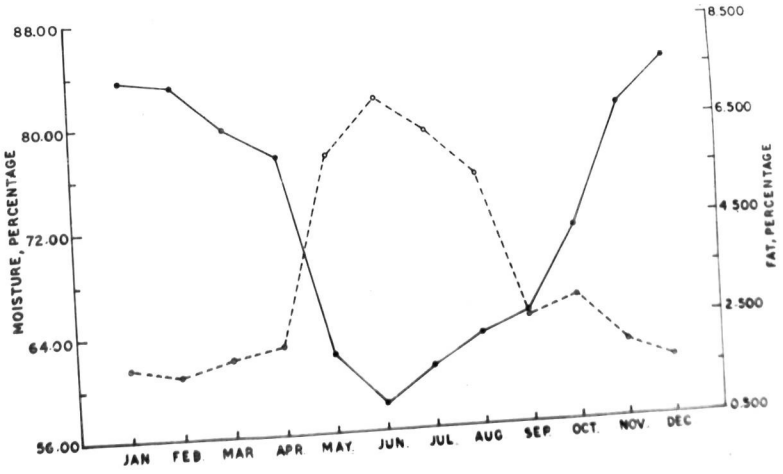
(b) Liver:

The amount of fat in the liver was much higher than in the muscle or the ovary and the variations in the fat content were more pronounced in different seasons of the year (Table 3, Fig. 7). Two peaks were also observed in the liver fat cycle. The first peak was recorded between April to June. This was followed by a rapid fall in July and August. The second peak of fat accumulation was observed in September. Unlike muscle, in liver the fat level remained fairly high during the winter months (January to March), though the lowest fat value was recorded in December. The highest fat value was observed in September. The cycles were almost similar in the two sexes.

The fat cycle of the liver did not show any definite relationship with that of the muscle except that high accumulation of fat in May or June and a low accumulation in December coincided in the two tissues.

Fig. 8 Seasonal variations in the moisture and fat contents of the ovary of H. fossilis.

●———, Moisture ; o-----, Fat.


88.00
80.00
72.00
64.00
56.00
MOISTURE, PERCENTAGE
8.500
6.500
4.500
2.500
0.500
FAT, PERCENTAGE
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

(c) Ovary:

The changes in the ovarian fat were more defined. The monthly fat values of the ovary have been given in Table 4 and plotted in Fig. 8. Peak accumulation of fat in the ovary was observed during May to August. This was followed by a rapid fall which continued till December when the lowest fat content was recorded. From January onwards the ovarian fat content again registered a rise. The ovary showed a more than three-fold increase in its fat content during a year (Table 4). As would be seen later, in ovarian tissue,protein was the more dominant component.

Thus, in all the three tissues of H. fossilis the lowest fat content was generally characteristic of the month of December.

II. Variations in moisture content:

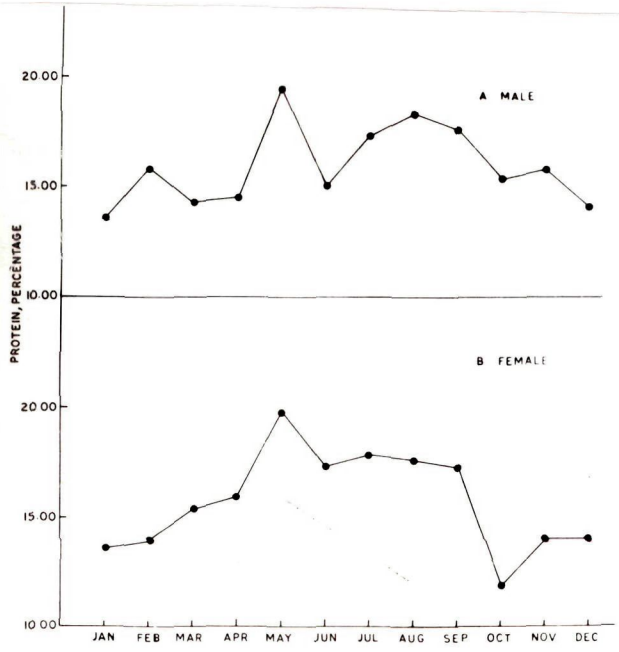
Moisture was found to show an inverse relationship with fat in all the three tissues, viz., muscle, liver and ovary of H. fossilis.

(a) Muscle:

Monthly values of moisture in the muscle have been given in Table 2 and plotted in Fig. 6. The muscle moisture values in a year ranged from about 75 % to 80 % in males and 71 % to 82 % in females.

The annual cycles of moisture in the muscle of both the sexes varied almost inversely with those of the fat.

Fig. 9 Seasonal variation in the protein content of the muscle of *H. fossilis*.


A MALE
B FEMALE
PROTEIN, PERCENTAGE
20.00
15.00
10.00
20.00
15.00
10.00
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

(b) Liver:

Liver generally contained relatively less moisture than the muscle, mainly because of a greater accumulation of fat in its tissue. Monthly values of moisture in liver have been given in Table 3 and represented in Fig. 7. It can be seen from the data that the inverse relationship between moisture and fat was more pronounced in liver than in the muscle. Thus, two peaks of fat accumulation corresponded with two distinct minima of moisture in the annual moisture cycles of male and female fishes.

(c) Ovary:

The variations in the moisture content were more clearly defined in the ovary than in the above two tissues. The values of moisture observed in the ovary in various months have been given in Table 4 and plotted in Fig. 8. The lowest values of moisture in the ovarian tissue were found in June and the highest in December. The inverse relationship between moisture and fat was also more distinct and better defined in the ovary.

III. Variations in protein content:

The protein constitutent of H. fossilis was greatly influenced by seasons. Definite phases of high and low protein values could be marked annually in all the three tissues (muscle, liver and ovary) of this species.

Fig. 10 Seasonal variation in the protein content of the liver of *H. fossilis*.

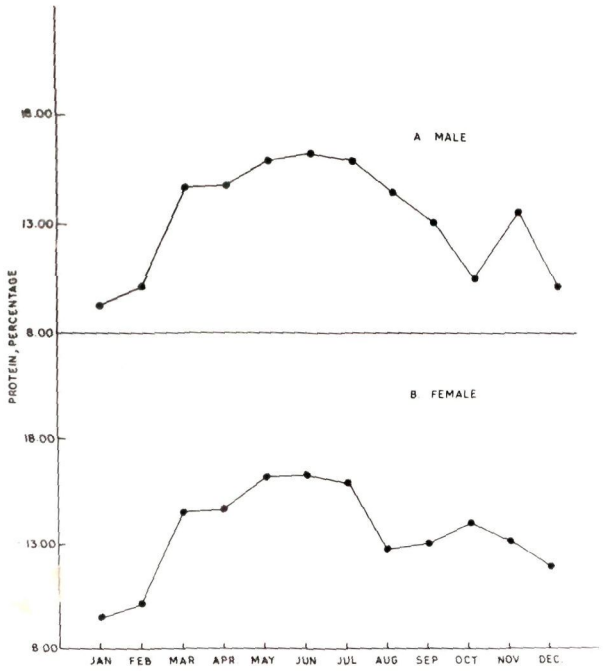
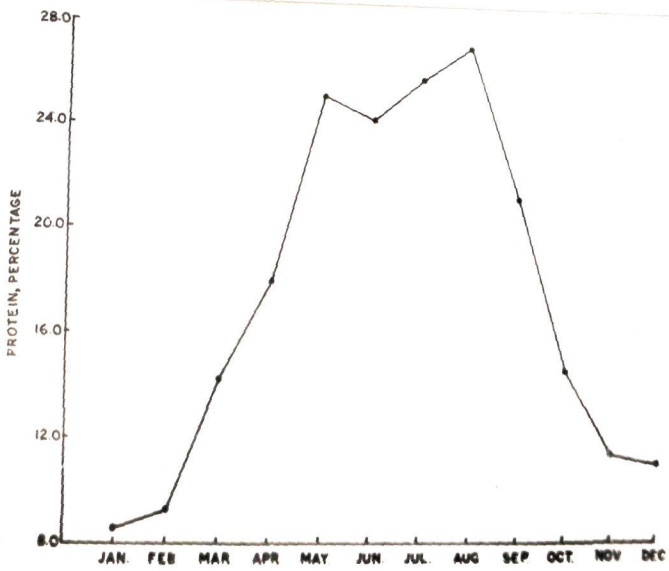

A MALE
B FEMALE
PROTEIN, PERCENTAGE
18.00
13.00
8.00
18.00
13.00
8.00
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

Fig. 11 Seasonal variation in the protein content of the ovary of *H. fossilis*.


28.0
24.0
20.0
16.0
12.0
8.0
PROTEIN, PERCENTAGE
JAN.
FEB
MAR
APR
MAY
JUN
JUL.
AUG
SEP
OCT.
NOV
DEC

(a) Muscle:

The muscle protein values did not register wide range of seasonal fluctuations. In a year, the range of variation was found to be from 11 % to 19 %, approximately (Table 5, Fig. 9). In female fishes the lowest protein percentage in the muscle was recorded in October but, thereafter, the protein values registered a rise till the maxima was obtained in May. A slight fall in the protein content occurred in June and the level maintained till about September. In males, on the other hand, the protein content was lowest in January but the peak was likewise obtained in May. The fall in the muscle protein content of male fishes in June was also greater than that of the females. Thus, in general, the protein value of the fish remained low during the winter months.

(b) Liver:

Well-defined seasonal variations were recorded in the liver protein content of the fish (Table 6, Fig. 10). The lowest protein percentage in the liver was recorded in January. From February onwards, the values registered a rise and the peak was obtained during May to June. The values of liver protein declined considerably in subsequent months. A slight increase in the protein percentages could again be seen in October and November in female and male fishes respectively.

(c) Ovary:

Monthly ovarian protein values have been given in Table 7 and plotted in Fig. 11. The variations observed in the protein content of the

Fig. 12 Seasonal variation in the ash content of the muscle of *H. fossilis*.

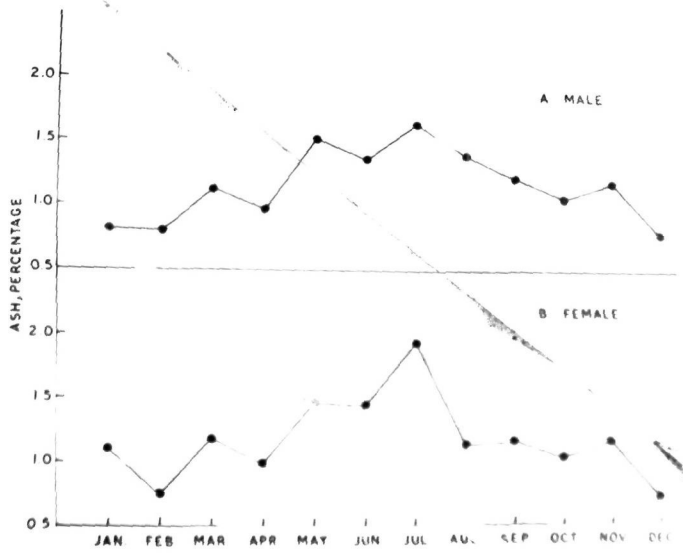

ASH, PERCENTAGE
2.0
1.5
1.0
0.5
A MALE
B FEMALE
JAN
FEB
MAR
APR
MAY
JUN
JUL
SEP
OCT
NOV

Fig. 13 Seasonal variation in the ash content of the liver of *H. fossilis*.

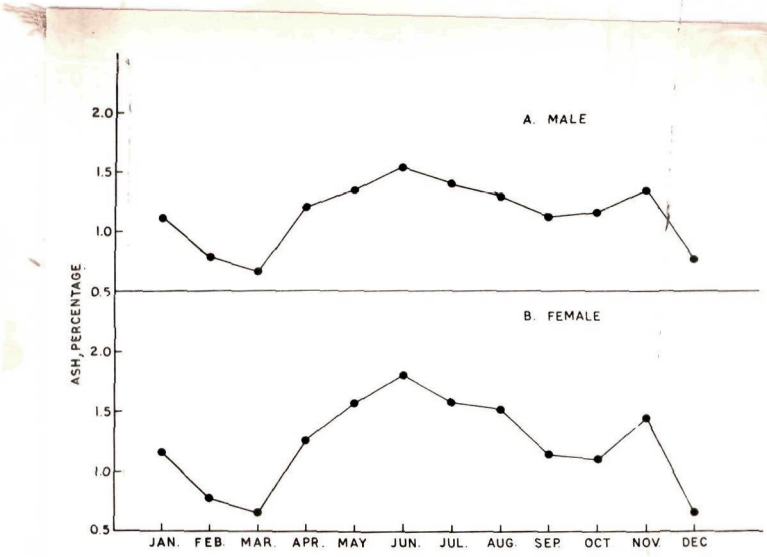

A. MALE
B. FEMALE
ASH, PERCENTAGE
2.0
1.5
1.0
0.5
JAN.
FEB.
MAR.
APR.
MAY
JUN.
JUL.
AUG.
SEP.
OCT
NOV.
DEC

Fig. 14 Seasonal variation in the ash content of the ovary of H. fossilis.

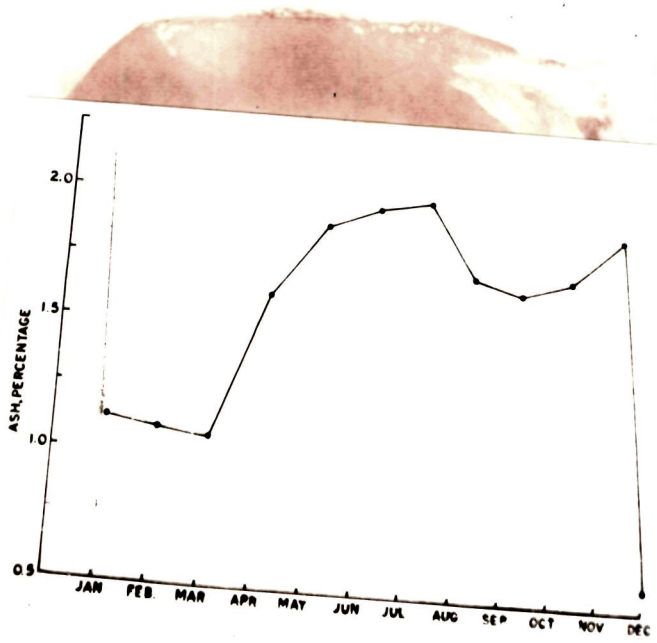

ASH, PERCENTAGE
2.0
1.5
1.0
0.5
JAN
FEB.
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

Fig. 15 Seasonal variation in the total cholesterol content of the muscle of H. fossilis.

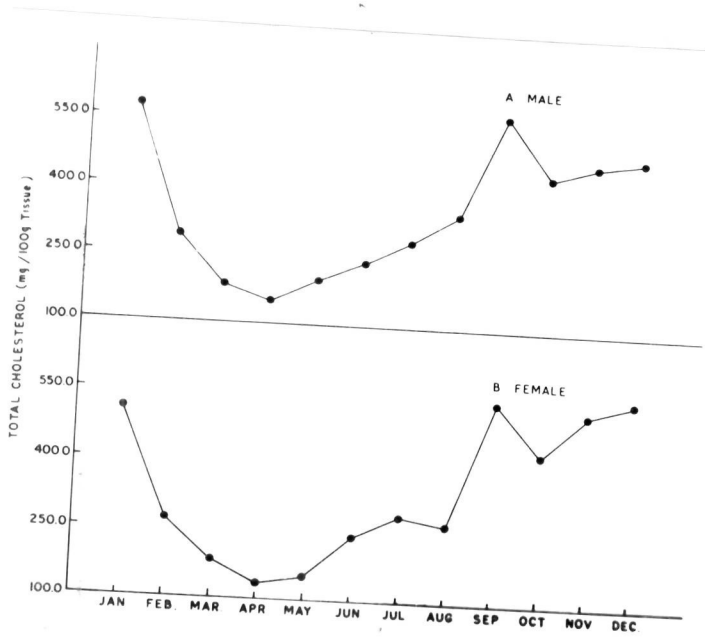
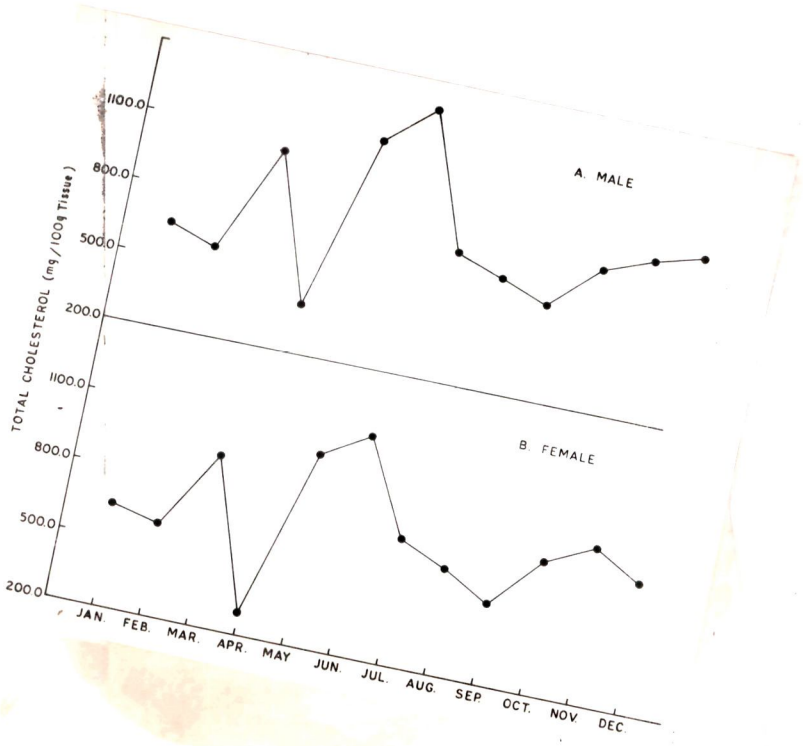

A MALE
B FEMALE
TOTAL CHOLESTEROL (mg/100g Tissue)
550.0
400.0
250.0
100.0
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

Fig. 16 Seasonal variation in the total cholesterol content of the liver of H. fossilis.


TOTAL CHOLESTEROL (mg/100g Tissue)
1100.0
800.0
500.0
200.0
A. MALE
1100.0
800.0
500.0
200.0
B. FEMALE
JAN.
FEB.
MAR.
APR.
MAY
JUN.
JUL.
AUG.
SEP.
OCT.
NOV.
DEC.

Declining values of cholesterol were noted from July to September. In later months (October-November) an increase in the cholesterol level was seen but diminution occurred from December or January to February. The values again rose in March. The cycles in the two sexes were almost identical, though the male fishes generally contained relatively more cholesterol in their liver.

(c) Ovary:

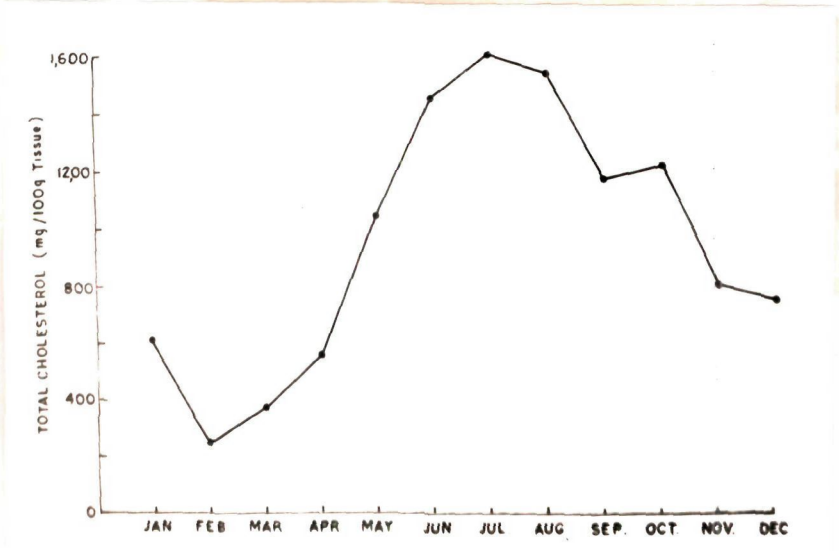
The values of total ovarian cholesterol observed in different months of the year have been given in Table 13 and plotted in Fig. 17. The ovarian cholesterol cycle was better defined. The ovary, in a year, accumulated greater quantity of cholesterol than the muscle or the liver. The cholesterol content in the ovary was lowest in February but increased gradually till the highest was obtained in July. A fall in the cholesterol level was, however, evident from August onwards.

Correlation between biochemical changes and the feeding and maturation cycles of the fish:

It has already been pointed out that the biochemical composition of fish tissue is greatly influenced by factors such as season, growth, feeding and maturation, etc. An attempt has been made to correlate the annual cycles of the various biochemical constituents of H. fossilis with two main features of its biology, namely, the feeding and maturation.

Gut content analyses of H. fossilis indicated that the fish relishes mainly on molluscs (gastropods), copepods, aquatic vegetation, algae,

Fig. 17 Seasonal variation in the total cholesterol content of the ovary of *H. fossilis*.


TOTAL CHOLESTEROL (mg/100g Tissue)
1,600
1200
800
400
0
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP.
OCT.
NOV.
DEC

decaying organic matter, sand, mud, etc. The adult and the larvae of certain insects, chironomid larvae, cypris and fish scales have also been recorded in the guts during certain months. The fish, however, showed no definite food preferences. Although no clear seasonal rhythm of feeding could be established, the fishes were generally found to indulge in more active feeding during certain summer and post-monsoon months. Feeding rate was maximum during September. Feeding was relatively low during winter (December-February). Seasonal changes in the gonad condition of H. fossilis were found to follow a well-defined cycle. Immature virgin fishes (stage I) occurred from about October to January. Recovering spent or maturing virgins (stage II) appeared in subsequent months till May. Whereas, ripening fishes (stage III) could be seen from the later half of May till June. The maximum percentages of ripe fishes (stage IV) were recorded during July and August. Spent fishes (stage V) started appearing from late August, their number reaching the maximum in September. Thus, the peak period of spawning appeared to be July-August. In an interesting paper on the biology of H. fossilis, Bhatt (1968) has made more or less similar observations on the feeding habit and spawning behaviour of this species.

The variations in the fat content of the various tissues of H. fossilis seemed related to feeding and maturation cycles of the fish. In certain parts of the year, these fluctuations appeared to be a net result of both feeding and maturation, as the effects of the two phenomena could not be clearly differentiated. Relatively high muscle

fat values observed during certain summer and post-monsoon months were found to coincide with the higher rate of feeding. Muscle fat content remained generally low during winter (December-March) when the feeding intake was low. Besides, the higher fat accumulation during May in males and during May-July in females was found to be coincide with the period when the fishes were passing through the ripe stage of gonad maturation. Similarly, a decline in the fat content of muscle in later months (August-October) characterized the spent and recovering phases of gonad maturation.

Many workers in the past have observed the effects of feeding, maturation or ripening on the fat content of fish muscle. The effect of food on the fat content of the Indian oil sardine, Sardinella longiceps, has been studied by Hornell and Naidu (1924) who attributed the variations in the fat content to the presence or absence of dinoflagellates and copepods in the diet. Similarly, Venkataraman and Chari (1951) attributed the peaks of fat accumulation in the muscle of the Indian mackerel, Rastrelliger kanagurta, to increased feeding activity. High muscle fat content in many other fishes during certain seasons of the year has also been correlated with intensive feeding and abundance of fish and planktonic food in the gut (Lovern and Wood, 1937; Wimpenny, 1938; Wilson, 1939; Sekharan, 1955; Ramaswamy, 1955; Vasavan et al., 1960; Jafri, 1968a, b, 1969; Jafri and Khawaja, 1968; Yagana, 1975).

Variations in the fat content of salmon during the spawning migration were observed by Atwater (1888) who found that the fish on its way to spawning grounds contained 13 % fat while the spent fishes contained only 3 % fat. Depletion of fat before spawning was observed in many species of herring (Johnstone, 1917; Channon and El-Saby, 1932; Lovern and Wood, 1937; Jensen, 1950). King salmon has been reported to store enormous amount of fat in its muscle before spawning which disappeared during the spawning migration as this fat formed the chief source of energy for the fish (Greene, 1919). In flounder, fat was generally withdrawn from the tissues during the maturation of gonads (Wilson, 1939). Hickling (1947) has also observed the effect of ripening on the muscle fat of pilchard. A correlation between the quantitative changes in the fat content and reproductive cycle was established by Black and Shwartz (1950) in the muscle of the South African pilchard. In ribbon-fish, Trichiurus haumela, a fall in the muscle fat content of the maturing groups during certain seasons of the year was related to the development of gonads (Sekharan, 1955). Idler and Bitners (1958), while investigating the biochemical changes in sockeye salmon during its spawning migration, observed a rapid fall in the fat values of the muscle with the development of genital products. Vasavan et al. (1960) have indicated that the rise and fall in the muscle fat content of the Indian oil sardine, S. longiceps, were due to the effect of the maturity of gonads. The influence of gonad maturation and spawning has earlier been observed on the muscle fat content of

certain cat-fishes by Jafri (1968b, 1969) and Yagana (1975).

In H. fossilis, the quantity of fat in the muscle was generally low and, therefore, the possibility of its contributing entirely towards the gonadal development seemed less convincing, though a clear depletion of the muscle fat could be seen with spawning.

There was a more clear correlation between the feeding activity and the liver fat cycle of the fish. Fat level in the liver remained low during winter (December-March) when feeding was less intensive. The peak periods of fat accumulation in summer (April-June) and in September were accompanied with active feeding.

Since liver appeared to be the main organ where a greater accumulation of fat occurred, its fat cycle also seemed to be affected by the maturation and depletion of gonads. Advancement in maturation was accompanied with an initial accumulation of fat in the liver which continued till May. The period of peak ripeness of gonad (July-August) was characterised by a rapid depletion of the liver fat reserves in both the sexes, presumably due to their utilization in gonad maturation and energy demands of the body. An immediate recovery of fat in the liver occurred in the post-spawning month (September) and, as stated earlier, it appeared to be the result of active indulgence in feeding by the spent fishes.

A marked falling off in fat content concurrently with the

maturation of genital products was also noted in the liver of herring (Bruce, 1924) and whiting (Bull, 1928). Channon and El-Saby (1932) recorded a rise in the percentages of different fatty acids of the liver of herring before spawning which was followed by a sharp fall during spawning. The observations of Noguchi et al. (1953) on mackerel indicated that the liver weight and the oil content were much effected by reproduction. In sockeye salmon the liver fat of both the sexes showed a decline during spawning migration (Idler and Bitners, 1960). Depletion of fat with the final stages of maturation and during spawning has been observed in the liver of several other freshwater fish species (Jafri, 1968a, 1969; Jafri and Khawaja, 1968).

The ovarian fat cycle of H. fossilis synchronized well with the cycle of maturation and spawning. Progression in maturation was accompanied with a rapid accumulation of fat in the ovarian tissue and consequently, the highest values of fat were observed at peak ripeness stage. A distinct fall in the ovarian fat in September and a gradual rise in later months characterized the spent and recovering phases, respectively. The rise and fall in the ovarian fat were also accompanied with a rise and fall in the weight of the ovaries.

Milroy (1908) has observed that the fat content in the ovaries of herring increases steadily until spawning occurs. Channon and El-Saby (1932) have also noted a rise in the amount of fatty acids of

gonads during the maturation of herring. In the red mullet, Upeneus indicus, gonads were found to gain fat steadily from stage I onwards to a maximum in stage III (Ramaswamy, 1955). The observations of Idler and Bitners (1960) on migrating sockeye salmon indicated that the increase in the total ovarian fat during the entire migration period was about 200 %. A high degree of relationship between the gonadal fat and maturity has earlier been pointed out in several freshwater teleostean species (Jafri, 1968a, b; Jafri and Khawaja, 1968).

There seemed to be no direct correlation between the moisture cycles of various tissues and the feeding or spawning in H. fossilis. Monthly variations in moisture were related more to fluctuations in fat and perhaps also to other organic constituents of the tissue. In general, however, the degree of hydration of the various tiesues of this species registered a rise in the spent and recovering spent phases of the gonad maturation. Paton (as quoted by Idler and Bitners, 1958) while commenting upon the water changes in the muscle of the migrating Salmo salar states: "It is this increase in the percentage of water of the flesh which maintains the weight of the fish per fish of the standard length, although the solids as a whole have diminished". Milroy (1908) has also observed an increase in the hydration of the muscle of the spent herring. Presumably, the depleted fats in fish tissue at this stage get replaced by large quantities of water. Similarly,

low moisture values in tissues like ovary, during ripe stages may be to accommodate more reserves.

protein cycles of various tissues of H. fossilis also appeared to be influenced both by feeding and maturation cycles of the fish. Low muscle protein values during winter may be because of reduced food intake and consequently a greater utilization of protein for energy requirements. Similarly, high muscle protein values during summer may be attributed to intensive feeding of proteinaceous diet by the fish. The level of protein in the muscle, however, synchronized more accurately with the cycle of maturation and depletion of gonad. A rise in the muscle protein values accompanied the advancement in maturation or ripening stages. A fall in the values in June, on the other hand, coincided with the peak ripeness and spawning.

In trout, it has been reported that protein in the muscle varies with the intensity of protein food consumed (McCay and Tunison, 1936). Jafri (1968b) has, however, reported that in the cat-fish Mystus seenghala, the muscle protein cycle does not seem to be influenced by feeding alone.

Though the effect of low food intake during winter and high during summer was evident on the liver protein levels of H. fossilis, the cycle of rise and fall appeared to synchronize more with the maturation rhythm of the fish. Accumulation of protein reserves in the liver accompanied the progression in gonad maturation and consequently, the

highest liver protein values could be noted in ripe and almost running fishes in June. Spawning resulted in a fall in the liver protein content and in the spent fishes the liver was found to be much more depleted of its protein reserves. This diminution of protein in liver may also indicate to the utilization of protein in meeting the fundamental nitrogen demands of the body. Like the fat content, a slight recovery in the liver protein values observed during October-November may be the result of active feeding.

The rise and fall in the ovarian protein, on the other hand, seemed related mainly to its cycle of maturation and depletion. A greater accumulation of protein in the ovary was found associated with maturation and ripeness. A gradual fall after August coincided with the spent stages. Though protein accumulation in the ovary during maturation and peak ripeness was much greater, there seemed to be no direct indication of any substantial mobilization of protein reserves from the muscle or the liver towards gonad building.

The king salmon was found to store an excess of protein in the ovaries during its spawning migration (Greene, 1921). Bruce (1924) observed a substantial rise in the ovarian protein of herring during the early phases of maturation. Krishnamoorthi (1958), while studying the distribution of free amino acids in the ovaries of certain fresh-water fishes, observed an increase in the number of amino acids during the final phases of maturation and this he attributed to the

increased protein metabolism of the fishes. A rise in the total ovarian protein during maturation has also been observed in many other fishes (Idler and Bitners, 1960; Jafri, 1968a, b, 1969; Jafri and Khawaja, 1968)

The variations in the ash content of various tissues were more clearly related to gonad maturation cycle, though intensive feeding during summer also resulted in increased tissue ash levels. Maximum ash values in all the tissues observed during May-July were found associated with the ripening and ripe stages. There was more rapid increase of ash in the ovarian tissue with the advancement in maturation. A fall in the ash content, similarly, characterized the spawning and post-spawning phases of gonad maturation. Higher ash values during maturation probably indicate to an enhanced mineral metabolism of the fish. Some correlation of ash with gonad maturation have earlier been pointed out in fish tissue (Jafri, 1968a, b, 1969; Jafri and Khawaja, 1968).

Although no direct relationship could be established between muscle cholesterol cycle and feeding, it was observed that muscle cholesterol values of H. fossilis declined with the advancement in maturation, reaching the lowest at peak ripeness. Higher muscle cholesterol values characterised the spent and recovering spent stages.

Liver is known to be the chief organ where active synthesis of

cholesterol occurs. The rise and fall in the liver cholesterol levels of H. fossilis were found to correlate with feeding, maturation and spawning. Relatively high cholesterol content observed in the liver during summer and post-monsoon months and declining values during winter months may be the result of high and low rate of feeding respectively. The highest liver cholesterol value in June, on the other hand, corresponded with the final phases of maturation. The liver cholesterol, however, declined considerably during peak ripeness (July-August), indicating to a greater utilization of cholesterol which may serve as a precursor of sex hormones (androgen and oestrogen) and of other steroid hormones. A recovery in the liver cholesterol was evident during the recovering spent phase (October-December) of gonad maturation.

There seemed to be little correlation between the ovarian cholesterol cycle and feeding. However, as one would expect, the cholesterol cycle of the ovary was found to synchronize well with its cycle of maturation and depletion. Ripening was associated with a rapid synthesis and accumulation of cholesterol in the ovary and the highest values of this substance in July coincided with the period of peak ripeness. A fall was associated with the onset of spawning in August which continued in subsequent months. During the recovering spent phase the ovary always contained less cholesterol. The effect of maturation on the ovarian cholesterol of fish has earlier been pointed out by Idler and Bitners (1958) and Siddiqi (1966c).

SUMMARY

Seasonal variations in the various biochemical constituents of muscle, liver and ovary were studied in the cat-fish, H. fossilis (Bloch.). The fat content of the muscle showed two peak periods of accumulation -- one during November and the other during May-July. Low muscle fat values were recorded from December to April. Liver was more rich in fat than the muscle or the ovary and it was also characterized by two distinct phases of high fat content -- in May and in September. The lowest value of fat in the liver was noted in December. In ovary the maximum fat content was observed in June and the minimum in December. Moisture in various tissues registered distinct variations from season to season, and these were found related inversely to the quantitative changes in the fat content. Protein content was also observed to be greatly influenced by seasons. In general,protein values were low during winter and high during summer. The variations in the ovarian protein were of a greater magnitude. In the three tissues analysed, the ash values were generally low during winter and high during summer or monsoon months. The ovary contained higher ash content than the muscle or the liver. The variations in cholesterol followed well-defined seasonal cycles in the three tissues and the trends were more or less identical to those of the fat.

The seasonal cycles of the various biochemical constituents in the three tissues of the fish seemed to be governed partly by feeding

and partly by the cycle of maturation and depletion of gonad. High and low values of fat and protein were found to coincide with high and low rate of feeding. There was a general build up of fat, protein and ash with ripening and a depletion with spawning. In some tissues, like liver, an immediate recovery of these constituents was seen soon after spawning. The extent of accumulation and diminution of these constituents was much greater in the ovarian tissue. The degree of hydration in the tissues increased in spent fishes. Cholesterol content, on the other hand, showed a decline in muscle and liver at peak ripeness, though in the ovary the progression in maturation resulted in a rapid increase in the quantity of this substance.

PART II

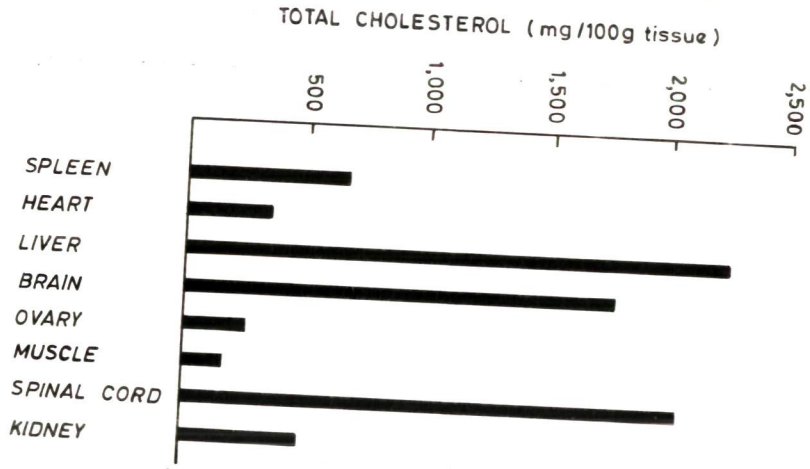
CHAPTER - III

DISTRIBUTION OF TOTAL CHOLESTEROL IN THE NORMAL ORGANS OF THE FRESHWATER MURREL, *OPHICEPHALUS PUNCTATUS* BLOCH.

INTRODUCTION

Though many workers in the past have investigated cholesterol content in a variety of fish species, reporting marked variations in the level of synthesis and distribution of this sterol in different organs (Idler and Bitners, 1958, 1960; Idler and Tsuyuki, 1958; Katada *et al.*, 1959, 1960; Siddiqi, 1966a,b,c; Love, 1970), the basis of these differences have been little explained from comparative and functional viewpoint. Moreover, related information on the freshwater fishes from tropical environment is far from complete. In an attempt to carry out further investigations on fish cholesterol, so as to fill up some gap in our knowledge on the subject, the present chapter describes the quantitative distribution of the total cholesterol in the various organs of the freshwater murrel, *Ophicephalus punctatus* Bloch., an important food fish of northern India.

Fig. 18 Histograms showing the distribution of total cholesterol in the normal organs of Ophicephalus punctatus Bloch.


TOTAL CHOLESTEROL (mg/100g tissue)
500
1,000
1,500
2,000
2,500
SPLEEN
HEART
LIVER
BRAIN
OVARY
MUSCLE
SPINAL CORD
KIDNEY

MATERIALS AND METHODS

Details of the methods of tissues sampling and cholesterol estimation were the same as described elsewhere (see under 'Procedure and Methodology').

RESULTS AND DISCUSSION

The values of the total cholesterol observed in the various organs of Q. punctatus have been given in Table 14 and represented in Fig. 18.

It would be evident from the data that the concentration of total cholesterol showed considerable variations from one organ to the other. The amount of cholesterol was lowest in the muscle and highest in the liver. It would be interesting to note that in this species the quantity of cholesterol in the liver was about 15 times higher than in the muscle. Other tissues showing fairly high amount of cholesterol were the brain and the spinal cord, while average values have been noted for the spleen, heart, kidney and ovary.

Earlier investigations on fish have indicated that the muscle, liver and ovary are rich in cholesterol (Siddiqi, 1966b).

The presence of significantly higher amount of total cholesterol in the liver of Q. punctatus points to the fact that, like other

animals, in this fish also the liver, being the main organ that performs key role in the various metabolic activities, is perhaps the chief site where actual synthesis of cholesterol occurs. Though cholesterol synthesis has been shown to take place in many other animal tissues such as skin, intestinal mucosa, adrenals, kidneys, brain and testis (West and Todd, 1967), the liver plays a dual role of regulating both the plasma cholesterol level and the total body cholesterol. Such a process might be operative in fish as well. It may be mentioned that the synthesis of cholesterol in the liver of O. punctatus has already been indicated to be controlled by a kind of feedback mechanism related to the internal absorption of cholesterol (Siddiqi, 1966a). In addition, a large proportion of metabolised cholesterol may be converted to bile acids in the liver which, in turn, might have a chain of consecutive feedback controlled processes. The liver cholesterol, along with phospholipids and specific proteins, may be involved in the formation of lipoproteins. Thus, liver may play a very active role in regulating the cholesterol metabolism.

A higher amount of cholesterol observed in the nervous tissues, like the brain and spinal cord of fish also seems to have some functional significance which would be discussed in the subsequent section.

Though cholesterol is an important component of gonads as a precursor of sex hormones, the presence of a low quantity of cholesterol

observed in the ovary of Q. punctatus (Table 14) may be indicative of a low rate of synthesis of this substance in the ovarian tissue, particularly during the period of sampling (October-November) for the present study. A visual examination of the ovary of the specimens analysed indicated that the ovary belonged to the recovering spent phase of maturation. Presumably, the rate of synthesis and the amount of cholesterol in the ovary increases with the advancement of maturation.

In other tissues, like muscle, kidney, spleen and heart, of Q. punctatus as well the rate of cholesterol synthesis appears to be low. The cholesterol in these tissues may subserve the general metabolic purposes usually assigned to this substance.

SUMMARY

The distribution of total cholesterol has been quantitatively studied in the various organs of the freshwater murrel, Q. punctatus Bloch. Significant variations have been observed in the amount of total cholesterol from one organ to the other. Liver, brain and spinal cord have been found to be fairly rich in cholesterol. Muscle tissue was found to have the lowest quantity of this substance. The significance of the observed distribution has been briefly discussed.

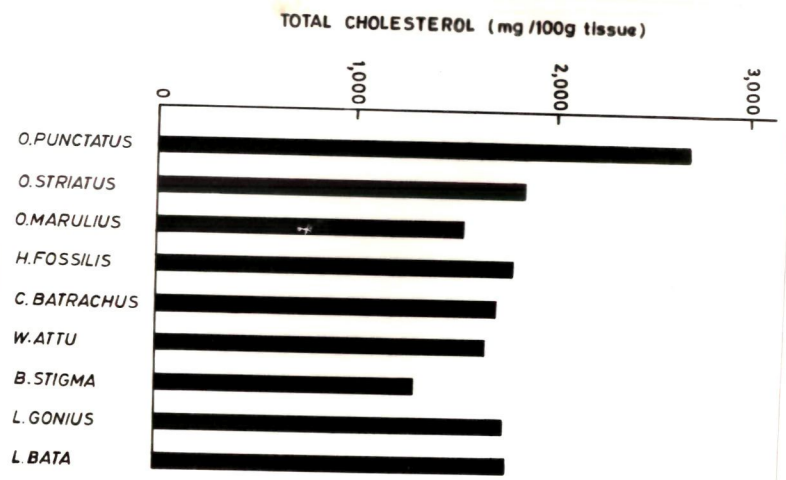
CHAPTER - IV

TOTAL CHOLESTEROL CONTENT OF THE BRAIN AND SPINAL CORD OF SOME FRESHWATER TELEOSTS

INTRODUCTION

The largest proportion of lipids of the central nervous system in vertebrates, including that in fish, is known to be present in the myelin sheath. Cerebrosides, cholesterol and sphingomyelin are the chief lipids of this sheath (West and Todd, 1967). Though several analyses have been made in the past of the various lipids of brain and spinal cord of vertebrates (McColl and Rossiter, 1952 a,b; Kabara, 1965; Cuzner and Davison, 1968; Rawlins and Smith, 1971; Ramsey et al., 1971 a,b; Martha and Davison, 1972; Smith, 1973), related studies on fish are limited (Ogino and Konno, 1950; Ananichev, 1961). Joshi and Magar (1955) have reported the distribution of lipids, including cholesterol, of a large number of Indian marine fish species. In this chapter data are presented for the comparative distribution of total cholesterol in the brain and spinal cord of certain freshwater fish species of northern India, representing several taxonomic groups.

Fig. 19 Histograms showing the distribution of total cholesterol in the brain of some freshwater teleosts.


TOTAL CHOLESTEROL (mg /100g tissue)
0
1,000
2,000
3,000
O.PUNCTATUS
O.STRIATUS
O.MARULIUS
H.FOSSILIS
C.BATRACHUS
W.ATTU
B.STIGMA
L.GONIUS
L.BATA

MATERIALS AND METHODS

Methods of sampling, tissue preparation and cholesterol estimation were the same as described under 'Procedure and Methodology' (pages 10-18).

RESULTS AND DISCUSSION

The mean concentration of total cholesterol in the brain and spinal cord of the various species analysed have been given in Table 15 and plotted in Figs. 19 and 20 respectively. The highest concentration of total cholesterol was observed in the brain and the spinal cord of the murrel, Ophicephalus punctatus and the lowest in that of the carp, Barbus stigma. On an average basis, the murrels were found to contain the highest quantity of total cholesterol in their brain, the cat-fishes came next while the carps were the poorest in this respect. The same group-wise pattern of cholesterol distribution was evident in the spinal cord of the various fish species analysed (see Fig. 20).

It is of interest to note that in all the nine species studied, the spinal cord was characterized by a much higher concentration of cholesterol than the brain. McColl and Rossiter (1952 a,b), while making a comparative study of the lipids in the brain and spinal cord of a representative series of vertebrates, including fishes, also

Fig. 20 Histograms showing the distribution of total cholesterol in the spinal cord of some freshwater teleosts.

observed that the percentage of certain lipids and cholesterol was much higher in the spinal cord.

Another interesting point which has emerged from the present study was that species with low total cholesterol content in their nervous tissues, like the carp, were mainly herbivorous, while those with higher values, such as the cat-fishes and murrels, were mainly carnivorous. This indicates towards the possible influence of diet on the total cholesterol concentration of nervous tissues in fish. Similarly, the concentration of total cholesterol was greater in species which are generally more active, like the active-air-breathing murrel, Q. punctatus. Though Love (1970) has pointed out that cholesterol content in the blood serum is higher in active fishes, this characteristic for nervous tissues require further confirmation.

The small standard deviation for the concentration of total cholesterol in the brain and spinal cord of most fish species analysed, however, indicate that, despite a considerable species-to-species variation, the concentration of this lipid in the nervous tissues of different individuals of the same species remain quite similar (Table 15). These findings are in accord with the results reported by other workers (McColl and Rossiter, 1952a, b).

Cholesterol, as poor conductor of electricity and with its dielectric value, is known to be a good insulator against electric discharge (West and Todd, 1967). Possibly, as an abundant constituent

of brain and spinal cord, as is apparent from the present study, it functions as insulating covering of impulse generating and transmitting structure. Occurrence of a greater concentration of cholesterol observed in the spinal cord may perhaps be to permit more rapid ionic conduction of impulses which are known to be electrical in characteristic.

SUMMARY

The distribution of total cholesterol has been studied in the brain and spinal cord of some freshwater teleosts. The cholesterol content was highest in the nervous tissues of the active carnivorous murrels but was relatively low in herbivorous carps. The spinal cord of all the species analysed contained much more concentration of total cholesterol than the brain. The pattern of distribution of this substance in the two tissues seemed species specific. The functional significance of cholesterol in the nervous tissues has been pointed out.

CHAPTER - V

CHOLESTEROL CONTENT IN THE EGGS OF SOME FRESHWATER TELEOSTS

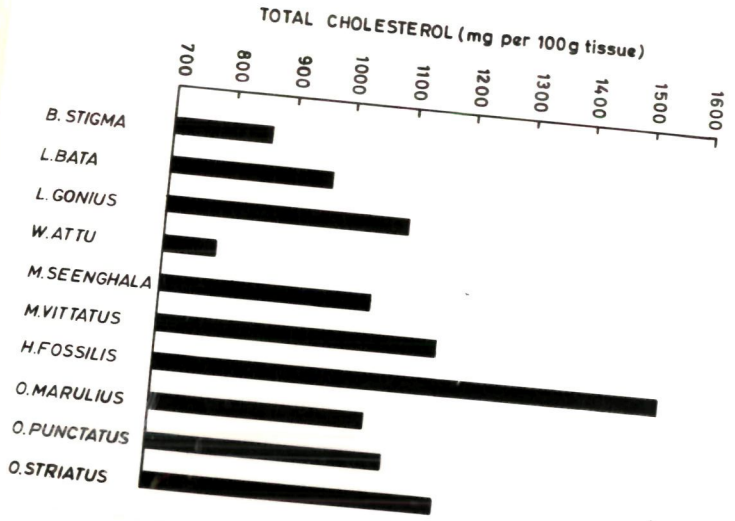
INTRODUCTION

Although the chemical analysis of the fish egg and ovary has been pursued by several workers in the past (Smith, 1957; Hasan and Jafri, 1964; Love, 1970), little attention has been paid to study the pattern of cholesterol distribution in hard roes and eggs. Earlier investigations on fish cholesterol in this laboratory were related to feedback mechanism in its biosynthesis in liver (Siddiqi, 1966a), distribution in various tissues (Siddiqi, 1966b), and relationship with feeding, maturation and spawning of fish (Siddiqi, 1966c; Siddiqui and Naseem, 1971). The present account deals with the distribution of total cholesterol in the ripe, unspawned eggs of some freshwater teleosts.

MATERIALS AND METHODS

Details of the method of egg sampling and the estimation of cholesterol have been described earlier under 'Procedure and Methodology'

Fig. 21 Histograms showing the distribution of total cholesterol in the ripe, unspawned eggs of some freshwater teleosts.


TOTAL CHOLESTEROL (mg per 100g tissue)
700
800
900
1000
1100
1200
1300
1400
1500
1600
B. STIGMA
L.BATA
L. GONIUS
W.ATTU
M.SEENGHALA
M.VITTATUS
H.FOSSILIS
O.MARULIUS
O.PUNCTATUS
O.STRIATUS

(pages 10-18).

RESULTS AND DISCUSSION

The values obtained for the total cholesterol content in the eggs of various species analysed have been summarized in Table 16 and represented in Fig. 21. The fish egg, with its relatively bulky yolk, has been found to be fairly rich in cholesterol. The cholesterol values in the eggs have, however, been found to differ from one species to another. The lowest value (790 mg/100 g) was recorded in the eggs of Wallago attu and the highest(1537 mg/100 g) in those of Heteropnuestes fossilis. On an average basis, however, the amount of cholesterol, with the exception of W. attu, have been found to be higher in the eggs of cat-fishes and murrels than in those of the carps. It may be interesting to point out that the nervous tissues of carps were also found to be poorest in their cholesterol content (see page 50). It may be mentioned that the eggs of some of the species, like Ophicephalus punctatus, were reported to be rich in lipids (Hasan and Jafri, 1964), but no general relationship could be established between the concentration of lipid and total cholesterol contents in fish eggs. Similarly, no definite correlation could be noted between egg diameter and the total egg cholesterol in the various fish species examined.

The synthesis of cholesterol, a precursor of sex and other

steroid hormones, is known to take place in many animal tissues, including the ovary, and the egg yolk is specially rich in this substance (West and Todd, 1967; Love, 1970). The variations observed in the amount of egg cholesterol in various teleosts may presumably be linked to the differences in the initial rate of cholesterol synthesis that occur during maturation in the ovarian tissue. Ripening of oocytes is perhaps associated with a greater mobilization and synthesis of cholesterol in ovarian tissue and by the time the eggs are fully mature, a considerable quantity of this substance is laid down in the egg yolk. Surprisingly enough, no relationship could be found to exist between the cholesterol content of eggs and the fecundity of the various species examined.

It is, however, interesting to observe that fishes which are generally carnivorous, consuming more fatty or proteinaceous diet, like the cat-fishes and murrels, contained relatively more concentration of cholesterol in their eggs than the herbivorous carps which generally relish on plant food. Though a part of cholesterol in animal's body is known to arise from the diet it consumes, it may require some more work to ascertain if diet has any bearing on the total egg cholesterol concentration in fish. At the moment, it may be presumed that the distribution of cholesterol in fish egg, in general, is species specific.

SUMMARY

The total cholesterol content has been estimated in the ripe, unspawned eggs of some freshwater teleosts. The eggs have been found to be fairly rich in cholesterol but showed variations from one species to another. The significance of the observed variations has been pointed out.

CHAPTER - VI

VARIATIONS IN THE TOTAL LIVER CHOLESTEROL LEVEL OF THE MAJOR CARP, *CIRRHINA MRIGALA* (HAM.) DURING MATURATION

INTRODUCTION

Sexual maturation is known to be accompanied by profound changes in the chemistry of fish. The depletive effects of maturation and spawning have been studied, in detail, in many fish species and marked variations have been observed in tissue constituents like amino-acids, glycogen, lipids and inorganic ions (Love, 1970). Though many authors in the past have also shown a relationship of serum and tissue cholesterol with maturation and egg production in fish (Channon and El-Saby, 1932; Idler and Tsuyuki, 1958; Idler and Bitners, 1960; Robertson et al., 1961a; McCartney, 1966, 1967), similar studies on Indian freshwater teleosts are relatively few (Siddiqi, 1966c; Siddiqui and Naseem, 1970). The depletive effect of gonad maturation on liver cholesterol level of the cat-fish, *H. fossilis*, has been pointed out in the earlier section (Chapter II). The present chapter deals with the changes in the total liver cholesterol level of a

Fig. 22 Variations in the total liver cholesterol content of Cirrhina mrigala (Ham.) during maturation.

common major carp, Cirrhina mrigala (Ham.), during its maturation cycle.

MATERIALS AND METHODS

Methods for the identification of gonad maturity stages, liver sampling and cholesterol estimations have been described under 'Procedure and Methodology' (pages 11-18).

RESULTS AND DISCUSSION

The total cholesterol content of the liver of C. mrigala at each maturity stage of the fish has been tabulated in Table 17 and plotted in Fig. 22. As would be evident from Fig. 22, a marked variation occurred in the liver cholesterol level of this species during the maturation cycle. The highest value of cholesterol in the liver was recorded when the fish was passing through the recovering phase of the gonad maturation. This was followed by a sharp and distinct depletion of liver cholesterol reserves and the minimal value was recorded during the ripe stage when the fish was almost ready for spawning. A corollary to this is also apparent in the observations on H. fossilis (page 42). On an average, a 59 % fall was recorded in the cholesterol reserve of the liver of C. mrigala from the recovering to ripe stage. A slight recovery in the cholesterol

content was, however, observed in the liver of the spent individuals. This recovery from the ripe to the spent phase was found only to be 38 %. The cycles in the two sexes were almost identical, though the cholesterol level in the liver of female fishes was relatively low. A distinct influence of gonad maturation on liver cholesterol level of C. mrigala was thus evident.

From these observations it appears that advancement in maturation brings about a mobilization of cholesterol reserves from liver towards gonad development and these may serve as the precursor of sex hormones (androgens and oestrogens) and of other steroid hormones. A steady loss of cholesterol from the liver and intestine and a rapid gain by the gonad in herring was observed earlier by Channon and El-Saby (1932). Siddiqui and Naseem (1970), while observing seasonal variations in the blood serum of C. mrigala, have similarly noted some influence of gonad maturation on the total serum cholesterol level of this fish. It may be worthwhile to add that a marked influence of gonad maturation has earlier been recorded on the proximate chemical composition of the tissues of C. mrigala and a clear possibility of the mobilization of liver-fat reserves towards gonadal development was pointed out (Jafri, 1968a). The observed changes in the cholesterol distributional pattern of liver of this fish may thus be related to changes in the cholesterol metabolism encountered during the maturation of the fish and necessitated, besides

other factors, by the demand for sex hormones.

The present findings on C. mrigala seem consistent with the observations of Idler and Bitners (1960) and Idler and Tsuyuki (1958) who have recorded a fall in the cholesterol content of serum, liver and gonad of both sexes of sockeye salmon, Oncorhynchus nerka, during maturation, which reached the minimal at the time of peak maturity. McCartney (1967) has also observed that serum cholesterol in Salmo trutta becomes minimal during the period of greatest sexual maturity. The cholesterol concentration patterns were thought, by these authors as well, to be related to changes in the cholesterol metabolism associated with the formation of sex hormones which have a steroid structure.

SUMMARY

Liver cholesterol level of Cirrhina mrigala (Ham.), an important major carp, has been found to be markedly influenced by the cycle of maturation and depletion of gonads. In both sexes, the highest amount of cholesterol was noted in the liver during the recovering phase. Advancement in maturation was accompanied by a depletion in the liver cholesterol reserve and the minimal value was obtained at the time of peak ripeness. These changes in the

concentration pattern of liver cholesterol seemed related to variations in the cholesterol metabolism of the fish, necessitated, besides other factors, by the demand for sex hormones.

CHAPTER - VII

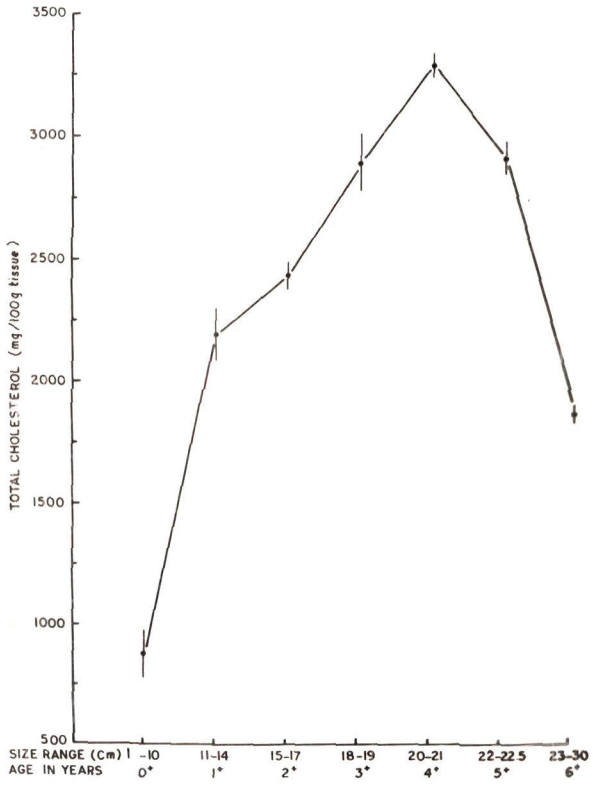
INFLUENCE OF AGE ON THE TOTAL LIVER CHOLESTEROL LEVEL OF FRESHWATER MURREL, *OPHICEPHALUS PUNCTATUS* BLOCH.

INTRODUCTION

There is a considerable body of literature on the chemical changes that occur in the animal tissue chemistry during development and growth. In fish, however, most studies on growth and aging are physiological or anatomical and chemical studies are relatively few (Love, 1970). Moreover, there is paucity of data on this aspect of tissue chemistry of tropical zone fish.

Though cholesterol has been estimated, besides other tissues, in the liver of many species of freshwater and marine fishes (Wilber, 1954; Idler and Bitners, 1960; Katada *et al.*, 1960; Ananichev, 1961; Zama, 1963; Shimma and Taguchi, 1964a,b; Siddiqi, 1966b; Suzuki, 1966), there seems to be no earlier evidence of the influence of age on the cholesterol content of fish liver. The present author, therefore, desired to investigate the influence of age on the total liver cholesterol level of *Ophicephalus punctatus* Bloch., a common

Fig. 23 Changes in the total liver cholesterol level of Ophicephalus punctatus with increasing age of the fish. Vertical lines express the standard error for the mean at each age point.


3500
3000
2500
2000
1500
1000
500
TOTAL CHOLESTEROL (mg/100g tissue)
SIZE RANGE (Cm) 1-10 11-14 15-17 18-19 20-21 22-22.5 23-30
AGE IN YEARS 0+ 1+ 2+ 3+ 4+ 5+ 6+

freshwater murrel, and the results have been reported in this chapter.

MATERIALS AND METHODS

The details of experimentation have been described under 'Procedure and Methodology' (pages 11-18).

RESULTS AND DISCUSSION

Liver cholesterol values observed in different age-groups of O. punctatus have been given in Table 18 and plotted in Fig. 23. 'P' values, based on students 't' test, between the year-classes 0^+ vs 1^+, 1^+ vs 2^+, 2^+ vs 3^+, 3^+ vs 4^+, 4^+ vs 5^+ and 5^+ vs 6^+, and their level of significance have been given in Table 19.

The values plotted in Fig. 23 indicate that the level of total cholesterol in the liver increased with increasing age up to a maximum when the fishes were 4 years old. The increase in cholesterol from 0^+ age-group fishes to the fishes of 4^+ age-group was about 47 %. However, this maximum attained by 4^+ age-group fishes, was not maintained with further increase in age, but registered a marked fall, reaching a low level in fishes of 6^+ years age.

While no earlier information is available on fish liver, it

has been reported that in carps there is a tendency for cholesterol in brain to increase with increase in body weight (Long, 1961) and size (Ogino and Konno, 1950). Das (1965) has indicated a parabolic relationship between the age and total cholesterol content in the blood of the major carp, Catla catla, whereas McCartney (1965) has not observed any influence of size increase on the total serum cholesterol of brown trout.

Presumably, the variations in the concentration of cholesterol, observed in the liver of O. punctatus, seem related to changes in cholesterol metabolism encountered during the aging of the fish and necessitated, besides other factors, by changes in growth pattern, diet, maturation and hormonal levels.

It has been pointed out earlier that O. punctatus, like many other tropical fishes, continues to grow in size and weight almost throughout its life, but the rate of annual growth falls down considerably in older fishes (Qasim and Bhatt, 1966). This decrease in the growth rate of older fish is reflected in its chemical composition (Khawaja and Jafri, 1967), and presently also in the cholesterol level of its liver. It may be of interest to mention that, in this particular species, the young fishes feed mostly on insect larvae, copepods, dephnids, etc., while the older ones become more piscivorous (Qayyum and Qasim, 1964). The changes in the

composition of diet from a less proteinaceous diet in younger fishes to a more proteinaceous diet in older ones might be an important additional factor in elevating the liver cholesterol level during growth, since it is known that a high protein diet reduces fat deposition but increases cholesterol ester content of the liver (Harper, 1967).

Many authors in the past have also shown a relationship between maturation, egg production, spawning and the serum and tissue cholesterol level of fish (Idler and Bitners, 1958; Idler and Tsuyuki, 1958; Robertson *et al.*, 1961a; McCartney, 1966, 1967). An interesting relationship between the liver cholesterol level and maturation has earlier been pointed out in the carp, *C. mrigala* (Chapter VI). It is interesting to note that in most fishes, including *O. punctatus*, the fecundity or the capacity of egg production increases with length, weight or age (Qayyum and Qasim, 1963) and the larger fishes are generally more depleted with spawning. Usually, however, the fecundity may fall down after certain age. It may, therefore, not be surprising to presume that the increase observed, in the total cholesterol level of liver in *O. punctatus*, with increasing age, and a fall in very old fish, may in part, be related to the sexual activity of the fish as also to the demand for sex hormones, as cholesterol is the parent steroid from which these hormones are derived. It may be added that the total serum and tissue cholesterol of this species had earlier been found to vary seasonally, mainly due to feeding conditions and maturation

cycle (Siddiqi, 1966c). It may thus be concluded that the changes in liver cholesterol level of O. punctatus presently observed may be a manifestation of aging and a net result of variations in growth rate, diet and sexual cycle of the fish.

SUMMARY

Influence of age has been investigated on the total liver cholesterol level of the freshwater murrel, Ophicephalus punctatus Bloch. The cholesterol content was markedly influenced by the age of the fish. The relative concentration of cholesterol registered a substantial rise with increasing age up to a maximum when the fish attained an age of 4^{+} years. A significant fall in the cholesterol level was, however, noticed beyond this age. The significance of the observed variations in the concentration pattern of liver cholesterol has been briefly discussed in the light of the known facts on the biology of this species.

CHAPTER - VIII

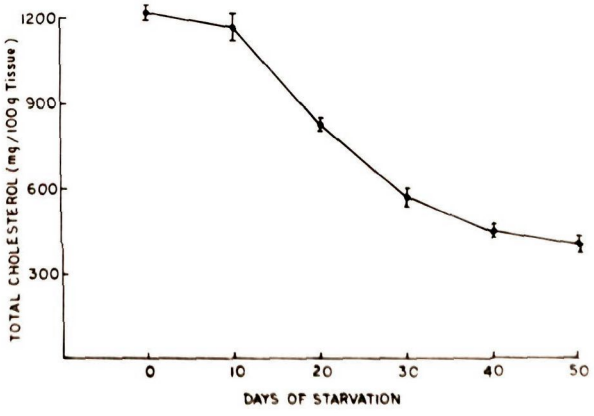
INFLUENCE OF STARVATION ON THE BRAIN AND LIVER CHOLESTEROL LEVELS OF THE CAT-FISH, HETEROPNUESTES FOSSILIS (BLOCH.)

INTRODUCTION

In all the animals, including fish, metabolism is accompanied by a series of complex reactions. Any cessation of the arrival of metabolites from the gut via the blood stream may cause marked changes in the chemistry and metabolism of the animal. A large number of studies have appeared on the changes in the chemistry of fish tissue, blood and serum during starvation (Greene, 1919; Love, 1958; Phillips et al., 1960; Scott, 1962; Creac'h and Serfaty, 1965; Inui and Ohshima, 1966; Kamara, 1966; Kosmina, 1966; Robertson et al., 1967; Love et al., 1968; Wilkins, 1967; Larsson and Lewander, 1973).

Cholesterol is the principal sterol of animal tissue and is perhaps the only member of this group occurring in food that is readily absorbed from the intestinal tract. Many organs are, however, able to synthesize cholesterol, and its rate of formation is regulated by the amount present in the diet (Schoenheimer and Breusch, 1933; Hotta et al.,

Fig. 24 Influence of starvation on the total liver cholesterol content of H. fossilis. Vertical lines express the standard error for the mean at each point.


1200
900
600
300
TOTAL CHOLESTEROL (mg/100g Tissue)
0
10
20
30
40
50
DAYS OF STARVATION

1954; Harper, 1967). Though a number of studies have been made in the past on the pattern of variations in the cholesterol content of animal tissue, blood and serum during short and prolonged starvations (White et al., 1959; Searcy, 1969; Kerpel et al., 1971), information on fish is far from complete.

The present chapter describes the influence of starvation on the total cholesterol levels of the brain and liver of the cat-fish, Heteropneustes fossilis (Bloch.).

MATERIALS AND METHODS

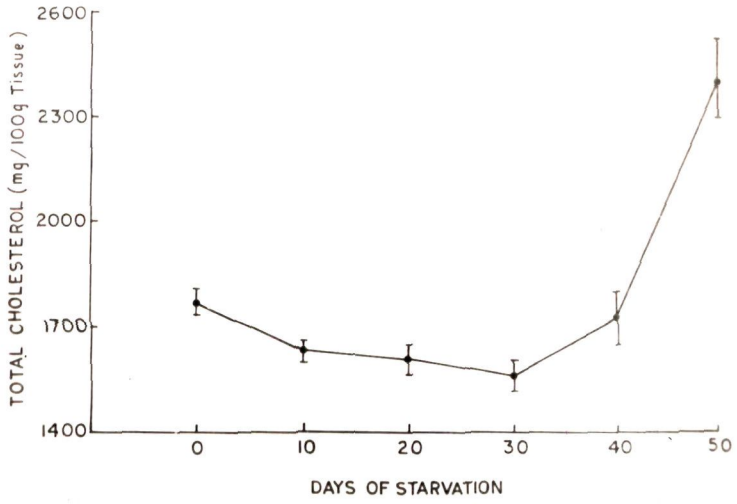
The details of the experimentation have been given under 'Procedure and Methodology' (pages 12-18).

RESULTS AND DISCUSSION

The values of total cholesterol in the liver and brain tissues of H. fossilis during different periods of starvation have been given in Table 20 and plotted in Figs. 24 and 25 respectively.

As would be evident from the data, starvation of the fish resulted in a marked fall in the total cholesterol content of the liver. In brain, however, the cholesterol level showed an initial decline up to 30 days of starvation but increased considerably

Fig. 25 Influence of starvation on the total brain cholesterol content of H. fossilis. Vertical lines express the standard error for the mean at each point.


TOTAL CHOLESTEROL (mg/100g Tissue)
2600
2300
2000
1700
1400
0
10
20
30
40
50
DAYS OF STARVATION

thereafter. The fall in the cholesterol content of the liver as a result of 50 days of starvation was about 67 %. The rise in the cholesterol content of the brain for the same period of starvation was recorded to be 31 %.

As the period of starvation progressed, a steady fall in the weight of the fishes also occurred. The decline in weight recorded for every 10 days interval during the entire period of starvation was of the order of 11.7, 13.1, 16.2, 19.2 and 20.9 %, respectively. On an average, the loss in the body weight of the fish was found to be 209.740 mg per day.

Though the fishes are known to be adapted for short period of starvation, longer periods may cause an imbalance and a major change in their metabolic economy (Love, 1970). Analyses of tissue glycogen of H. fossilis carried out earlier in this laboratory (Jafri, unpublished data) indicated that within a few days of starvation the glycogen level of the liver of this species was largely depleted and the content in the muscle fell to a minima. Presumably, after the loss of original glycogen stores during fasting, the fish becomes totally dependent upon the metabolism of triglycerides from the adipose tissue and amino acids derived from the tissue protein. It may be mentioned that during prolonged fasting due to the inhibition of lipogenesis the adipose tissue also indirectly becomes the main source of serum esterified fatty acids (Baker et al., 1968).

Since cholesterol in the brain is primarily of endogenous origin, the relatively low cerebral cholesterol level recorded in H. fossilis presumably indicate to a reduced synthesis of this sterol in the brain tissue. Cerebral cholesterol synthesis may, however, be altered either by metabolic inhibition of any one step in the intermediary pathway of cholesterol synthesis or by limitation in the availability of co-factors for the reduction of desmosterol to cholesterol (Shah, 1972).

As already mentioned, liver may well be the chief organ concerned with the regulation of total body content of cholesterol and with the control of plasma cholesterol levels. Besides supplying endogenous cholesterol and cholesterol esters to plasma, it governs the bile acid production from cholesterol.

Most cholesterol esters in the plasma are known to be formed by the action of lecithin cholesterol acyltransferase which utilizes fatty acids from the plasma lecithin and thus are also indirectly dependent on the fatty acid supply (Glomset and Ibid, 1968).

The later phases of starvation perhaps result in an increased oxidation of fatty acids by the liver with a resultant increased elaboration and production of acetoacetate and 3 hydroxy-butyrate and these compounds may become a major fuel for the brain. Since acetoacetate is the precursor of cholesterol, the cholesterol content

in brain should increase during the later stage of starvation, as is apparent from the present findings on H. fossilis. The liver thus occupies a central position in the metabolism of cholesterol as it does in the case of other lipids.

The finding that liver cholesterol level is decreased in fasted fish seems consistent with the observations of Slakey et al., (1972) and Johnson and Shah (1974) who found that the conversion of liver squalene to sterol (cholesterol) is reduced in starved rats. The depressing effect of fasting on the cholesterol biosynthesis of liver has also been pointed out earlier by several workers (West et al., 1966).

SUMMARY

Influence of starvation has been observed on the total cholesterol levels of the brain and liver of the cat-fish, H.fossilis (Bloch.). The cholesterol level decreased with starvation in liver but in brain, after registering an initial fall, it showed a distinct rise. The variations in the cholesterol level of fish observed during starvation have been attributed to the changes in the rate of cholesterol synthesis and metabolism.

CHAPTER - IX

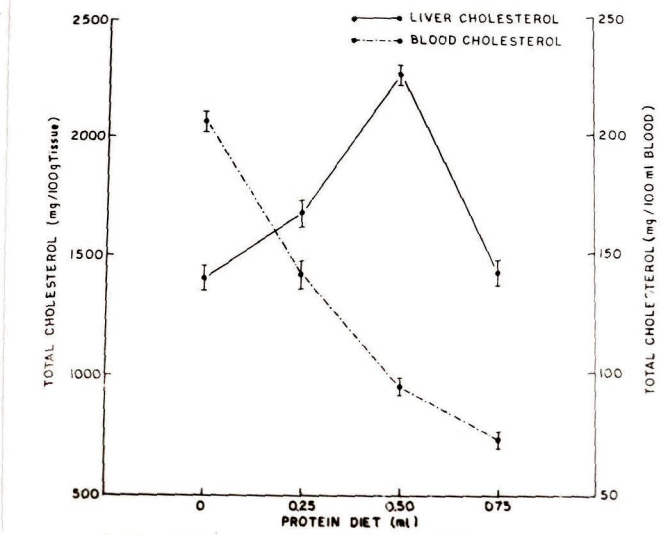
INFLUENCE OF DIET ON THE TOTAL BLOOD AND LIVER CHOLESTEROL LEVELS OF THE CAT-FISH, *CLARIAS BATRACHUS* (LINN.).

INTRODUCTION

The supply of cholesterol to body is derived in two ways: exogenous cholesterol viz. from the diet and endogenous cholesterol by synthesis in the tissues, particularly in liver (Wilson *et al.*, 1967). The quantity and composition of the diet is known to influence the rate of cholesterol synthesis and the latter appears to vary inversely with the quantity of cholesterol available from the exogenous sources (White *et al.*, 1959). Several studies have been made in the past to examine the effects of diets on the cholesterol level of various vertebrates (Dam, 1930; Schoenheimer and Breusch, 1933; Morris and Chaikoff, 1959; Nath *et al.*, 1959; White *et al.*, 1959; Cantarow and Schepartz, 1962; Beveridge *et al.*, 1963; Harper, 1967; West and Todd, 1967; Wilson *et al.*, 1967).

In the present studies an attempt has been made to examine the effects of three different diets, namely, protein, carbohydrate and

Fig. 26 Influence of protein diet on the total cholesterol content of the liver and blood of C. batrachus. Vertical lines express the standard error for the mean at each point.


LIVER CHOLESTEROL
BLOOD CHOLESTEROL
2500
2000
1500
1000
500
250
200
150
100
50
TOTAL CHOLESTEROL (mg/100gTissue)
TOTAL CHOLESTEROL (mg/100 ml BLOOD)
0
0.25
0.50
0.75
PROTEIN DIET (ml)

cholesterol (fat), on the cholesterol levels of the blood and liver tissue of the cat-fish, Clarias batrachus (Linn.).

MATERIALS AND METHODS

The experimental details for this study have been given elsewhere (see under 'Procedure and Methodology').

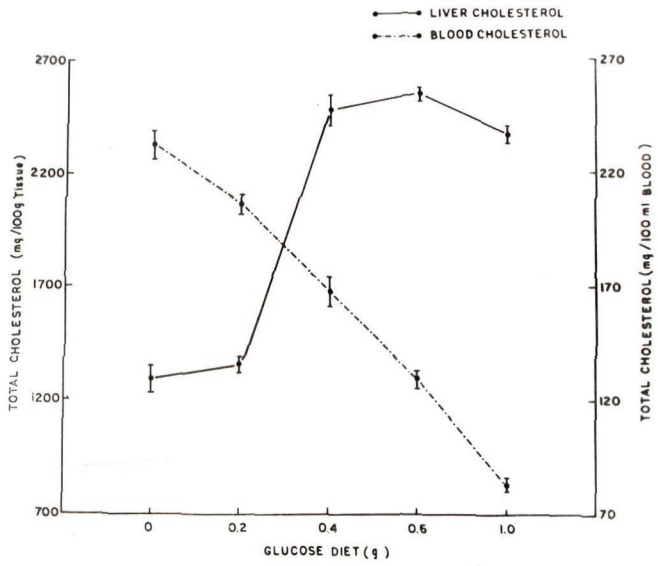
RESULTS AND DISCUSSION

The influence of protein, carbohydrate and cholesterol (fat) diets was evident on the total blood and liver cholesterol levels of C. batrachus. The values of cholesterol content obtained with various concentrations of these diets have been given in Tables 21-23 and plotted in Figs. 26-28.

As can be seen from Fig. 26, the blood cholesterol level of the fish showed a marked fall with increased uptake of protein diet. The fall in the blood cholesterol level was calculated to be about 65 %. The liver cholesterol level, on the other hand, registered an increase up to 0.50 ml concentration of the protein diet. This increase was recorded to be 61 %, approximately.

On a carbohydrate diet, the liver cholesterol level was also found to elevate up to a certain dose limit. The highest liver

Fig. 27 Influence of glucose diet on the total cholesterol content of the liver and blood of C. batrachus. Vertical lines express the standard error for the mean at each point.

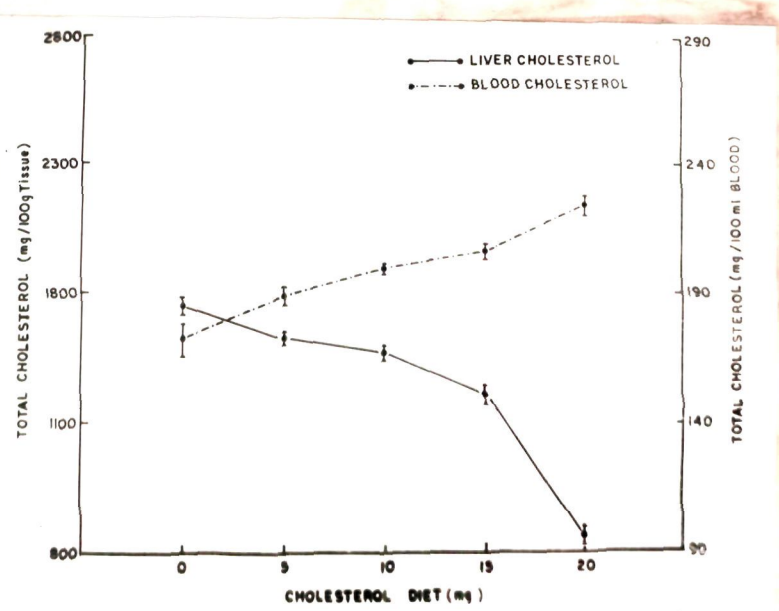

LIVER CHOLESTEROL
BLOOD CHOLESTEROL
TOTAL CHOLESTEROL (mg/100g Tissue)
TOTAL CHOLESTEROL (mg/100 ml BLOOD)
2700
2200
1700
1200
700
270
220
170
120
70
0
0.2
0.4
0.6
1.0
GLUCOSE DIET (g)

cholesterol content (2541.200 mg/100 g) was recorded when the fishes were fed with 0.6 g of the glucose diet but a marked fall occurred when the concentration of the dose was increased to 1.0 g (Fig. 27). The percentage increase in the liver cholesterol level registered up to 0.6 g glucose dose was about 97. It is, however, interesting to note that with increased uptake of carbohydrate diet, as with protein diet, the blood showed a fall in its cholesterol level, the percentage of fall being 64.

With increased uptake of cholesterol (fat) diet, the liver cholesterol level of the fish was found to decline considerably, whereas the blood cholesterol level showed a significant rise (Fig. 28). The decline in the liver cholesterol level was about 50 % while the rise in the blood cholesterol level was only about 30 %. The present observations thus showed an interesting inverse relationship between the liver and the blood cholesterol levels of C. batrachus under the influence of cholesterol diet.

A corollary to the present findings is apparent in the work of Morris and Chaikoff (1959) who observed a depression in the rate of cholesterol synthesis subsequent to excess cholesterol feeding. That a decreased liver cholesterol synthesis accompanies an elevated total blood cholesterol level has also been shown in several vertebrates (White et al., 1959; Cantarow and Schepartz, 1962; Harper, 1967; West and Todd, 1967). However, the influence of cholesterol diet on

Fig. 28 Influence of cholesterol diet on the total cholesterol content of the liver and blood of C. batrachus. Vertical lines express the standard error for the mean at each point.


LIVER CHOLESTEROL
BLOOD CHOLESTEROL
TOTAL CHOLESTEROL (mg/100g Tissue)
TOTAL CHOLESTEROL (mg/100 ml BLOOD)
2800
2300
1800
1100
800
290
240
190
140
90
0
5
10
15
20
CHOLESTEROL DIET (mg)

the blood level of this constituent seems uncertain (Wilson et al., 1967).

Many workers have reported that the rate of cholesterol synthesis increases on a high carbohydrate diet (White et al., 1959; West and Todd, 1967), and the present findings on C. batrachus provide additional support to their views. The low protein intake, on the other hand, has been reported to be hypercholesteremic (Nath et al., 1959; Beveridge et al., 1963).

As mentioned earlier, the liver plays a dual role of regulating both the plasma cholesterol level and the total body cholesterol, the latter being determined by a balance between the amount synthesized, plus that absorbed from the diet, and the amount converted to other substances, plus that excreted in the faeces. As the amount of cholesterol absorbed from the diet increases, the amount synthesized by the liver goes down and this may reach very low level, as is evident from the present observation on C. batrachus. Thus, synthesis of cholesterol in the liver appears to be controlled by a kind of feedback mechanism related to the internal absorption of cholesterol. The existence of a feedback mechanism in the cholesterol synthesis of fish has earlier been pointed out by Siddiqi (1966a). Besides, it is known that a large proportion of cholesterol metabolised in the liver is converted to bile acids through a chain of consecutive feedback

controlled processes.

The plasma cholesterol level also appears to vary with the quantities of blood phospholipids, triglycerides and, probably, specific proteins present in the plasma to combine with cholesterol as lipoproteins. Thus, both dietary and endogenous factors affect the distribution of cholesterol between plasma and the liver (West and Todd, 1967).

It may be mentioned that the endogenous component of cholesterol in a given tissue may arise, in certain situations, from smaller components or is transported to that tissue by plasma (Morris and Chaikoff, 1959).

The present findings of C. batrachus thus indicate that the liver not only processes dietary cholesterol but, in addition, is the principal source of synthetic component of plasma or blood cholesterol. To what extent the other tissues of the fish contribute to the plasma or blood cholesterol, could not be ascertained and point to the scope of future investigations in this direction.

SUMMARY

The total blood and liver cholesterol levels of C. batrachus, a freshwater cat-fish, showed a marked response to different types of

diets. The cholesterol level of the blood declined with an increased uptake of the carbohydrate and protein diets but showed a distinct rise with the cholesterol (fat) diet. The liver cholesterol level, on the other hand, registered an initial increase with the protein and carbohydrate diets, but declined substantially when higher doses of these diets were offered to the fishes. Increased intake of cholesterol diet induced an overall fall in the liver cholesterol level. An inverse relationship was noted between the liver and the blood cholesterol levels of the fishes fed on cholesterol diet. Evidence of a feedback mechanism operating in the liver of the fish was thus obtained.

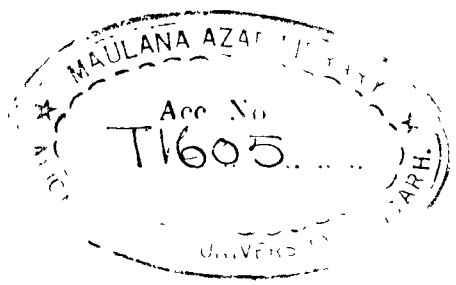


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TABLE - 1

Fat, water, protein and ash percentages in the muscle of the various body zones of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Body zones	Fat %	Water %	Protein %	Ash %
Dorsal series	1	0.496 ± 0.030	83.043 ± 0.355	13.774 ± 0.296	1.239 ± 0.032
	2	0.725 ± 0.055	81.856 ± 0.356	14.586 ± 0.194	1.269 ± 0.011
	3	0.975 ± 0.022	80.490 ± 0.135	15.270 ± 0.083	1.303 ± 0.128
Ventral series	4	0.629 ± 0.054	82.446 ± 0.065	13.754 ± 0.385	1.245 ± 0.036
	5	0.795 ± 0.534	80.380 ± 0.062	14.506 ± 0.257	1.296 ± 0.234
	6	1.191 ± 0.518	79.790 ± 0.051	15.353 ± 0.083	1.304 ± 0.094

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TABLE - 2

Monthly mean values of fat and moisture in the muscle of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Months	Fat percentage		Moisture percentage	
		Male	Female	Male	Female
1974	Feb.	0.400	0.650	80.085	80.470
	Mar.	0.422	0.565	80.630	80.080
	Apr.	0.441	0.425	80.354	80.770
	May	1.400	1.055	75.890	76.234
	Jun.	0.550	1.073	79.440	76.330
	Jul.	0.560	1.101	77.770	76.010
	Aug.	0.480	0.721	80.040	79.470
	Sept.	0.607	0.583	77.900	78.960
	Oct.	0.505	0.625	79.690	80.125
	Nov.	1.507	1.827	75.100	70.993
	Dec.	0.478	0.430	78.402	82.645
1975	Jan.	0.565	0.645	79.855	80.125

TABDE - 3

Monthly mean values of fat and moisture in the liver of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Months	Fat percentage		Moisture percentage	
		Male	Female	Male	Female
1974	Feb.	6.454	6.765	74.150	74.280
	Mar.	6.332	6.781	74.980	71.740
	Apr.	10.000	9.988	71.530	72.634
	May	11.790	11.123	70.000	69.735
	Jun.	7.600	10.000	72.025	72.030
	Jul.	3.680	2.850	78.450	77.090
	Aug.	4.032	3.207	75.560	76.470
	Sept.	12.961	12.000	70.320	72.890
	Oct.	7.326	2.576	72.100	73.231
	Nov.	7.317	6.265	72.158	71.297
	Dec.	2.212	1.920	75.590	75.995
1975	Jan.	7.110	9.083	73.015	72.058

TABLE - 4

Monthly mean values of fat and moisture in the ovary of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Months	Fat %	Moisture %
1974	Feb.	1.718	83.140
	Mar.	2.000	79.760
	Apr.	2.199	77.451
	May	5.880	61.906
	Jun.	6.963	58.110
	Jul.	6.300	60.790
	Aug.	5.438	63.180
	Sept.	2.678	64.760
	Oct.	2.957	71.295
	Nov.	2.033	80.937
	Dec.	1.676	84.556
1975	Jan.	1.880	83.615

TABLE - 5

Monthly mean values of protein in the muscle of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Months	Protein percentage	
		Male	Female
1974	Feb.	15.912	13.918
	Mar.	14.325	15.437
	Apr.	14.520	15.912
	May	19.475	19.771
	Jun.	15.181	17.337
	Jul.	17.337	17.871
	Aug.	18.406	17.604
	Sept.	17.601	17.337
	Oct.	15.437	11.934
	Nov.	15.759	14.071
	Dec.	14.101	14.071
1975	Jan.	13.596	13.596

TABLE - 6

Monthly mean values of protein in the liver of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Months	Protein percentage	
		Male	Female
1974	Feb.	10.120	10.153
	Mar.	14.590	14.516
	Apr.	14.780	14.606
	May	15.912	16.150
	Jun.	16.230	16.387
	Jul.	15.912	15.912
	Aug.	14.490	12.765
	Sept.	13.181	13.032
	Oct.	10.509	14.071
	Nov.	13.621	13.126
	Dec.	10.153	11.934
1975	Jan.	9.203	9.559

TABLE - 7

Monthly mean values of protein in the ovary of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Months	Protein percentages (on wet weight basis)
1974	Feb.	9.203
	Mar.	14.248
	Apr.	18.020
	May	25.293
	Jun.	24.422
	Jul.	26.131
	Aug.	27.490
	Sept.	21.493
	Oct.	14.754
	Nov.	11.459
	Dec.	11.103
1975	Jan.	8.490

TABLE - 8

Monthly mean values of ash in the muscle of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Months	Ash percentage	
		Male	Female
1974	Feb.	0.800	0.739
	Mar.	1.123	1.179
	Apr.	0.991	0.980
	May	1.520	1.463
	Jun.	1.380	1.450
	Jul.	1.660	1.940
	Aug.	1.410	1.130
	Sept.	1.230	1.157
	Oct.	1.080	1.039
	Nov.	1.210	1.162
	Dec.	0.808	0.711
1975	Jan.	0.819	1.105

TABLE - 9

Monthly mean values of ash in the liver of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Months	Ash percentage	
		Male	Female
1974	Feb.	0.780	0.778
	Mar.	0.668	0.652
	Apr.	1.215	1.271
	May	1.350	1.578
	Jun.	1.530	1.790
	Jul.	1.400	1.580
	Aug.	1.280	1.520
	Sept.	1.121	1.147
	Oct.	1.160	1.117
	Nov.	1.357	1.446
	Dec.	0.775	0.663
1975	Jan.	1.115	1.176

TABLE - 10

Monthly mean values of ash in the ovary of the cat-fish Heteropnuestes fossilis (Bloch.)

	Months	Ash percentage
1974	Feb.	1.093
	Mar.	1.067
	Apr.	1.630
	May	1.915
	Jun.	2.000
	Jul.	2.040
	Aug.	1.760
	Sept.	1.715
	Oct.	1.781
	Nov.	1.966
	Dec.	0.590
1975	Jan.	1.125

TABLE - 11

Monthly mean values of total cholesterol in the muscle of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Months	Cholesterol (mg/100 g wet tissue)	
		Male	Female
1974	Feb.	288.400	272.700
	Mar.	184.900	184.900
	Apr.	150.800	137.800
	May	197.400	153.550
	Jun.	241.300	241.300
	Jul.	288.400	288.400
	Aug.	350.200	272.600
	Sept.	576.800	542.300
	Oct.	445.000	429.300
	Nov.	476.400	523.400
	Dec.	489.000	551.300
1975	Jan.	576.800	510.500

TABLE - 12

Monthly mean values of total cholesterol in the liver of the cat-fish, Heteropnuestes fossilis (Bloch.)

	Months	Cholesterol (mg/100 g wet tissue)	
		Male	Female
1974	Feb.	576.000	592.400
	Mar.	1030.400	924.625
	Apr.	412.000	288.400
	May	1153.500	1009.400
	Jun.	1325.900	1134.600
	Jul.	749.100	730.000
	Aug.	680.000	648.800
	Sept.	610.400	539.000
	Oct.	802.400	764.800
	Nov.	877.600	858.800
	Dec.	934.000	749.100
1975	Jan.	639.400	639.400

TABLE - 13

Monthly mean values of total cholesterol in the ovary of the cat-fish, *Heteropnuestes fossilis* (Bloch.)

	Months	Cholesterol (mg/100 g wet tissue)
1974	Feb.	241.300
	Mar.	366.700
	Apr.	551.700
	May	1031.300
	Jun.	1435.600
	Jul.	1579.800
	Aug.	1516.400
	Sept.	1153.425
	Oct.	1194.300
	Nov.	783.600
	Dec.	730.300
1975	Jan.	607.700

TABLE - 14

Distribution of total cholesterol in the normal organs of the murrel, *Ophicephalus punctatus* Bloch.

Organs	Mean total cholesterol (mg/100 g wet tissue)
Spleen	677.33 ± 42.53
Heart	358.66 ± 25.33
Kidney	495.33 ± 30.44
Liver	2280.00 ± 89.66
Brain	1798.66 ± 64.31
Spinal cord	2084.00 ± 64.79
Ovary	276.00 ± 11.32
Muscle	184.66 ± 26.16

± SE

TABLE - 15

Mean concentration of total cholesterol in the brain and spinal cord of some freshwater fishes

Species	Total cholesterol (mg/100 g wet tissue)			
	Brain	SD	Spinal cord	SD
MURRELS				
1. *Ophicephalus punctatus* Bloch.	2683.333	36.01	4848.000	1.90
2. *Ophicephalus striatus* Bloch.	1840.000	10.00	3883.333	2.44
3. *Ophicephalus marulius* Ham.	1535.000	5.000	3761.666	2.86
CAT-FISHES				
4. *Heteropnuestes fossilis* (Bloch.)	1783.333	3.05	4457.333	7.74
5. *Clarias batrachus* (Linn.)	1705.333	3.03	4634.666	2.83
6. *Wallago attu* (Bl. & Schn.)	1648.333	2.86	3544.000	26.11
CARPS				
7. *Barbus stigma* (Cuv. & Val.)	1300.666	7.02	3650.666	3.26
8. *Labeo gonius* (Ham.)	1737.333	20.52	3941.333	35.85
9. *Labeo bata* (Ham.)	1740.666	1.05	3880.000	20.00

TABLE - 16

Mean concentration of total cholesterol in the eggs of some freshwater teleosts.

Species	Max. egg diameter (mm)	Total Cholesterol (mg/100 g wet tissue)
CARPS		
1. *Barbus stigma* (Cuv. & Val.)	0.653	864.00
2. *Labeo bata* (Ham.)	1.010	970.00
3. *Labeo gonius* (Ham.)	1.340	1103.00
CAT-FISHES		
4. *Wallago attu* (Bl. & Schn.)	1.316	790.00
5. *Mystus seenghala* (Sykes)	1.163	1049.00
6. *Mystus vittatus* (Bloch.)	0.626	1164.00
7. *Heteropnuestes fossilis* (Bloch.)	1.102	1537.00
MURRELS		
8. *Ophicephalus marulius* Ham.	1.554	1054.00
9. *Ophicephalus punctatus* Bloch.	1.049	1090.00
10. *Ophicephalus striatus* Bloch.	1.268	1182.00

TABLE - 17

Total cholesterol content in the liver of Cirrhina mrigala (Ham.) during different maturity stages.

Maturity stages	Mean Cholesterol (mg/100 g wet tissue)	
	Male	Female
Recovering	496.52 ± 23.77	460.58 ± 21.38
Ripening	376.48 ± 2.65	337.58 ± 5.39
Ripe	148.60 ± 3.77	140.00 ± 3.73
Spent	205.00 ± 6.23	186.20 ± 6.25

± SE

TABLE - 19

'P' values between the various year-classes with their level of significance in the murrel, Ophicephalus punctatus Bloch.

Year-classes	'P' Values
0^+ vs 1^+	>0.05 (significant at 5% level)
1^+ vs 2^+	<0.10 (ns)
2^+ vs 3^+	<0.10 (ns)
3^+ vs 4^+	<0.10 (ns)
4^+ vs 5^+	>0.10 (significant at 1% level)
5^+ vs 6^+	>0.02 (significant at 2% level)

(ns) - not significant

TABLE - 20

Effect of starvation on the total liver and brain cholesterol levels of the cat-fish, Heteropnuestes fossilis (Bloch.)

Starvation period (days)	LIVER				BRAIN			
	Cholesterol (mg/100 g tissue)	SE	'r'	Variance	Cholesterol (mg/100 g tissue)	SE	'r'	Variance
0	1216.00	25.43	3.61	1936.00	1771.33	37.75	2.13	1425.36
10	1161.00	51.00	7.59	7785.32	1629.33	29.48	3.13	2601.32
20	820.00	21.96	4.63	1444.00	1612.66	44.77	4.80	6001.29
30	563.33	30.15	9.26	2721.33	1564.00	42.77	4.73	5476.00
40	444.00	18.49	7.20	1024.00	1732.00	78.43	7.83	18411.99
50	396.00	27.16	11.86	2209.00	2322.66	112.14	8.35	37641.31

SE - Standard error

'r' - Coefficient of variation

TABLE - 21

Effect of protein diet on the total blood and liver cholesterol levels of the cat-fish, Clarias batrachus (Linn.)

Conc. of diet (ml.)	BLOOD				LIVER			
	Cholesterol (mg/100 ml)	SE	'r'	Variance	Cholesterol (mg/100 g tissue)	SE	'r'	Variance
0	206.340	3.64	3.05	39.690	1399.333	44.56	5.50	5942.773
0.25	141.786	4.45	5.43	59.427	1669.200	49.04	5.08	7198.360
0.50	94.700	2.52	6.01	19.147	2253.866	36.70	2.81	4032.253
0.75	72.740	2.52	6.01	19.147	1417.866	44.56	5.43	5942.773

SE - Standard error

'r' - Coefficient of variation

TABLE - 22

Effect of glucose diet on the total blood and liver cholesterol levels of the cat-fish, Clarias batrachus (Linn.)

Conc. of diet (g)	BLOOD				LIVER			
	Cholesterol (mg/100 ml)	SE	'r'	Variance	Cholesterol (mg/100 g tissue)	SE	'r'	Variance
0	232.773	5.26	4.35	102.875	1289.333	52.83	7.08	8354.173
0.20	205.980	3.45	2.90	35.760	1347.866	25.37	3.25	1927.239
0.40	167.653	5.95	6.14	106.123	2474.333	58.91	4.11	10386.653
0.60	128.893	2.68	3.60	21.622	2541.200	23.02	1.56	1586.994
1.00	83.046	2.30	4.80	15.920	2363.533	36.24	2.65	3931.292

SE - Standard error

'r' - Coefficient of variation

TABLE - 23

Effect of cholesterol diet on the total blood and liver cholesterol levels of the cat-fish, Clarias batrachus (Linn.)

Conc. of diet(mg)	BLOOD				LIVER			
	Cholesterol (mg/100 ml)	SE	'r'	Variance	Cholesterol (mg/100 g tissue)	SE	'r'	Variance
0	172.753	5.33	5.32	84.694	1744.600	27.35	2.71	2238.756
5.0	188.693	3.06	2.81	28.196	1623.000	15.01	1.60	674.996
10.0	199.113	0.88	0.77	2.358	1570.466	19.89	2.19	1184.247
15.0	206.860	2.72	2.28	22.296	1409.800	27.03	3.31	2186.993
20.0	224.140	3.64	2.81	39.221	858.800	23.64	2.75	1673.554

SE - Standard error

'r' - Coefficient of variation

GENERAL SUMMARY

The present work includes some interesting biochemical studies on the freshwater teleosts. The quantitative distributional patterns of fat, water, protein and ash was investigated in the flesh from different body locations of the common cat-fish, H. fossilis (Bloch). The ventral aspect of the body showed more accumulation of fat and ash than the dorsal aspect, though this dorso-ventral gradation was not evident in the protein content. The fat, protein and ash contents were also found to register an increase from the anterior to the posterior zones, both in the ventral as well as dorsal regions, of the body. The distribution of water followed an almost opposite pattern, indicating an inverse relationship with the fat and ash contents.

The various biochemical constituents of the muscle, liver and ovary of the above species showed distinct variations from season to season. The fat content of the muscle showed two peak periods of accumulation -- one during November and the other during May-July. Liver was more rich in fat than the muscle or the ovary and it was also characterized by two distinct phases of high fat content -- in May and September. In ovary, the maximum fat content was observed in June.

The lowest values of fat in both liver and ovary were observed in December. Variations in moisture content of the three tissues were found related inversely to the quantitative changes in the fat content. Protein and ash values were generally low during winter and high during summer or monsoon months. The variations in the tissue cholesterol were more or less identical to those of the fat.

The seasonal cycles of the various biochemical constituents in the three tissues of H. fossilis seemed to be governed partly by feeding and partly by the cycle of maturation and depletion of gonad. High and low values of fat and protein generally synchronized with high and low rates of feeding. There was a general build up of fat, protein and ash with ripening and a depletion with spawning. The extent of accumulation and diminution of these constituents was much greater in the ovarian tissue. The degree of hydration in tissues increased in spent fishes. Cholesterol content showed a decline in muscle and liver at peak ripeness, though in the ovary, progression in maturation resulted in a rapid increase in the quantity of this substance.

Some experimental studies were also made on fish cholesterol. In O. punctatus, a freshwater murrel, significant variations have been observed in the quantitative distribution of total cholesterol from one organ to the other. Liver, brain and spinal cord were fairly rich in cholesterol, while the muscle tissue was the poorest in this respect.

The total liver cholesterol level of this species was found to be markedly influenced by the age of the fish. A substantial rise in the concentration was evident with increasing age up to a maximum when the fish attained an age of 4^{+} years but significant fall occurred beyond this age. These changes were thought to be a manifestation of aging and a net result of variations in growth rate, diet and sexual cycle of the fish.

A comparative quantitative study of the distribution of total cholesterol in the brain, spinal cord and eggs of some freshwater teleosts indicated that the cholesterol content was highest in the nervous tissues of the active murrels but was relatively low in herbivorous carps. The spinal cord of all the species analysed contained more concentration of cholesterol than the brain. The ripe, unspawned teleostean eggs were also found to be fairly rich in this substance, though quantitative variations were observed from one species to another. The distribution of cholesterol in the nervous tissues and the eggs, in general, seemed species specific.

Liver cholesterol level of the major carp, C. mrigala, has been found to be markedly influenced by the cycle of maturation and depletion of its gonad. The highest value of cholesterol was noted during the recovering phase. Advancement in maturation was accompanied by a depletion in the liver cholesterol and the minimal value was reached at the time of peak ripeness. These changes in the concentration pattern of liver cholesterol seemed to be related to variations in the

cholesterol metabolism of the fish, necessitated, besides other factors, by the demand for sex hormones.

The cholesterol level of fish was significantly influenced by starvation and showed a marked response to different types of diets. In H. fossilis, the cholesterol level decreased with starvation in liver but in brain, after registering an initial fall, it showed a distinct rise. These changes have been attributed to the changes in the rate of cholesterol synthesis and metabolism. The cholesterol level of the blood of another cat-fish, C. batrachus, declined with an increased uptake of the carbohydrate and protein diets but showed a distinct rise with cholesterol (fat) diet. The liver cholesterol level of this species, on the other hand, registered an initial increase with the protein and carbohydrate diets, but declined substantially when higher doses of these diets were offered to the fishes. Increased intake of cholesterol diet induced an overall fall in the liver cholesterol level. An inverse relationship was noted between the liver and the blood cholesterol levels of the fishes fed on cholesterol diet. An evidence of a feedback mechanism operating in the liver of C. batrachuc was thus obtained.